Figure 42

Figure 42: An alignment of the IFN-gamma nucleotide sequences from human, cat, rodent species.

```
                                            1                                                50
AF081502 Marmota monax IFN-gamma      (1)   --------------------GGCCTAACTCTCTCTGAAACGATGAAATAC
   D30619 Felis catus IFN-gamma       (1)   CTACTGATTTCAACTTCTTTGCCCTAACTCTC--CGAAACGATGAATTAC
   X87308 Homo sapien IFN-gamma       (1)   --------------------------------------------------

                                            51                                              100
AF081502 Marmota monax IFN-gamma     (31)   ACAAGTTTATTTCTTGGCTTTCAGCTCTGCATCATTTTGGGTTCTTCTAG
   D30619 Felis catus IFN-gamma      (49)   ACAAGTTTTATTTTCGCTTTCCAGCTTTGCATAATTTTGTGTTCTTCTGG
   X87308 Homo sapien IFN-gamma       (1)   --------------------------------------------------

                                            101                                             150
AF081502 Marmota monax IFN-gamma     (81)   CTGTTACTCCCAGGACACAGTTAATAAACAAATAGAAGTTTTATTAGGAT
   D30619 Felis catus IFN-gamma      (99)   TTATTACTGTCAGCCCATGTTTTTTTAAACAAATACAAGCTTAAAGCGAT
   X87308 Homo sapien IFN-gamma       (1)   -TGTTACTGCCAGGACCCATATGTAAAAGAAGCAGAAAACCTTAAGAAAT

                                            151                                             200
AF081502 Marmota monax IFN-gamma    (131)   ATTTTAATGCAAGTAATTCAAAAGTATTCAGATGGCCCGTCTATCTGGTG
   D30619 Felis catus IFN-gamma     (149)   ATTTTAATGCAAGTAATCCAGATGTAGCAGATGGTGGGTCGCTTTTCGTA
   X87308 Homo sapien IFN-gamma      (50)   ATTTTAATGCAGGTCATTCAGATGTAGCGGATAATGGAACTCTTTTCTTA

                                            201                                             250
AF081502 Marmota monax IFN-gamma    (181)   GATATTTTGGATAAATGGAAAGAGGAGAGTGACAAAAAAGTAATCCAGAG
   D30619 Felis catus IFN-gamma     (199)   GACATTTTGAAGAACTGGAAAGAGGAGAGTGATAAAACAATAATTCAAAG
   X87308 Homo sapien IFN-gamma     (100)   GGCATTTTGAAGAATTGGAAAGAGGAGAGTGACAGAAAAATAATGCAGAG

                                            251                                             300
AF081502 Marmota monax IFN-gamma    (231)   CCAAATTGTCTCTTTCTACTTCAAACTCTTTGAACACTTAAAAAGCAA--
   D30619 Felis catus IFN-gamma     (249)   GCAAATTGTCTCCTTCTACCTGAAAATGTTTGAAAACCTGAAAGATGATG
   X87308 Homo sapien IFN-gamma     (150)   CCAAATTGTCTCCTTTTACTTCAAACTTTTTAGAAACTTTAAAGATGA--

                                            301                                             350
AF081502 Marmota monax IFN-gamma    (279)   -CAAGATCATCCAAAGGAGCATGGACACCATCAAGGGGGATCTTTTTGCT
   D30619 Felis catus IFN-gamma     (299)   ACCAGCGCATTCAAAGGAGCATGGACAGCATCAAGGAAGACATACTCGAT
   X87308 Homo sapien IFN-gamma     (198)   -CCAGAGCATCCAAAAGAGTGTGGAGACCATCAAGGAAGACATGAATGTC

                                            351                                             400
AF081502 Marmota monax IFN-gamma    (328)   AAGTTCTTCAACAGCAGTACCAATAAGGTGCAGGACTTCCTAAAGCGCTG
   D30619 Felis catus IFN-gamma     (349)   AAGTTGTTAAATACCAGGTCCAGTATAACGGATCAGCTTCCTCAAGCGAT
   X87308 Homo sapien IFN-gamma     (247)   AAGTTTTTCAATAGCAACAAAAAGAAACGAGATGACTTCGAAAAGCTGAC

                                            401                                             450
AF081502 Marmota monax IFN-gamma    (378)   TCAAGTTCAGGTAAATGACCTGAAGATCCAGCGTAAAGCAGTGAGTGAAG
   D30619 Felis catus IFN-gamma     (399)   TCAAATCCCTGTGAATGATCTCCAGGTCCAGCGCAAAGCAATAAATGAAG
   X87308 Homo sapien IFN-gamma     (297)   AATTATTCGGTAACTGACTTGAATGTCCAACGCAAAGCAATACATCAAC

                                            451                                             500
AF081502 Marmota monax IFN-gamma    (428)   TCAAGAAAGTCAATGAAATGATCTCTTTAGCACACTCTCCCTAAGGAGCA
   D30619 Felis catus IFN-gamma     (449)   TCTTCAAACTCATCAATCAGATCTCACCAAGATCTAACCTGAGGAAGCGG
   X87308 Homo sapien IFN-gamma     (347)   TCATCCAAGTGATGGCTGAACTGTCGCCAGCAGCTAAAACAGGGAAGCGA

                                            501                                             550
AF081502 Marmota monax IFN-gamma    (478)   AAAAGGAGTCAGTCTTCGATTGGGCCTGGACAGCATCCAAATAACAGTTC
   D30619 Felis catus IFN-gamma     (499)   AAAAGGAGCCAGAATGTGTTCGAGGCCGGTAGAGCATGGAAATAATGCTT
   X87308 Homo sapien IFN-gamma     (397)   AAAAGGAGTCAGATGCTGTTTCGAGGTCGAAGAGCATCCCAG--------

                                            551             569
AF081502 Marmota monax IFN-gamma    (528)   CTGATGCCTGC--------
   D30619 Felis catus IFN-gamma     (549)   GTCCTGCCTGCAATATTTG
   X87308 Homo sapien IFN-gamma     (439)   -------------------
```

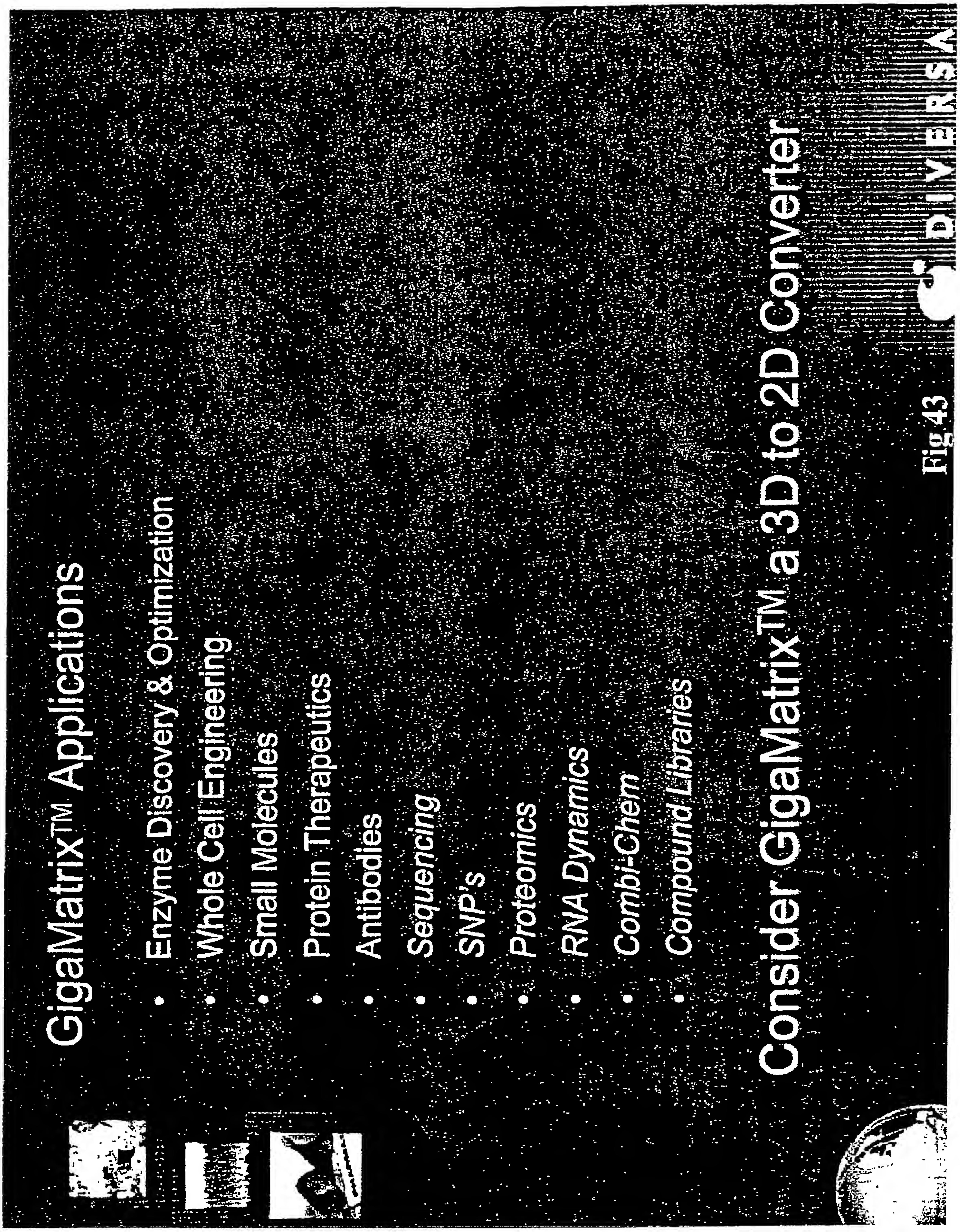
GigaMatrix™ Applications
• Enzyme Discovery & Optimization
• Whole Cell Engineering
• Small Molecules
• Protein Therapeutics
• Antibodies
• Sequencing
• SNP's
• Proteomics
• RNA Dynamics
• Combi-Chem
• Compound Libraries
Consider GigaMatrix™ a 3D to 2D Converter
DIVERSA

Fig 43

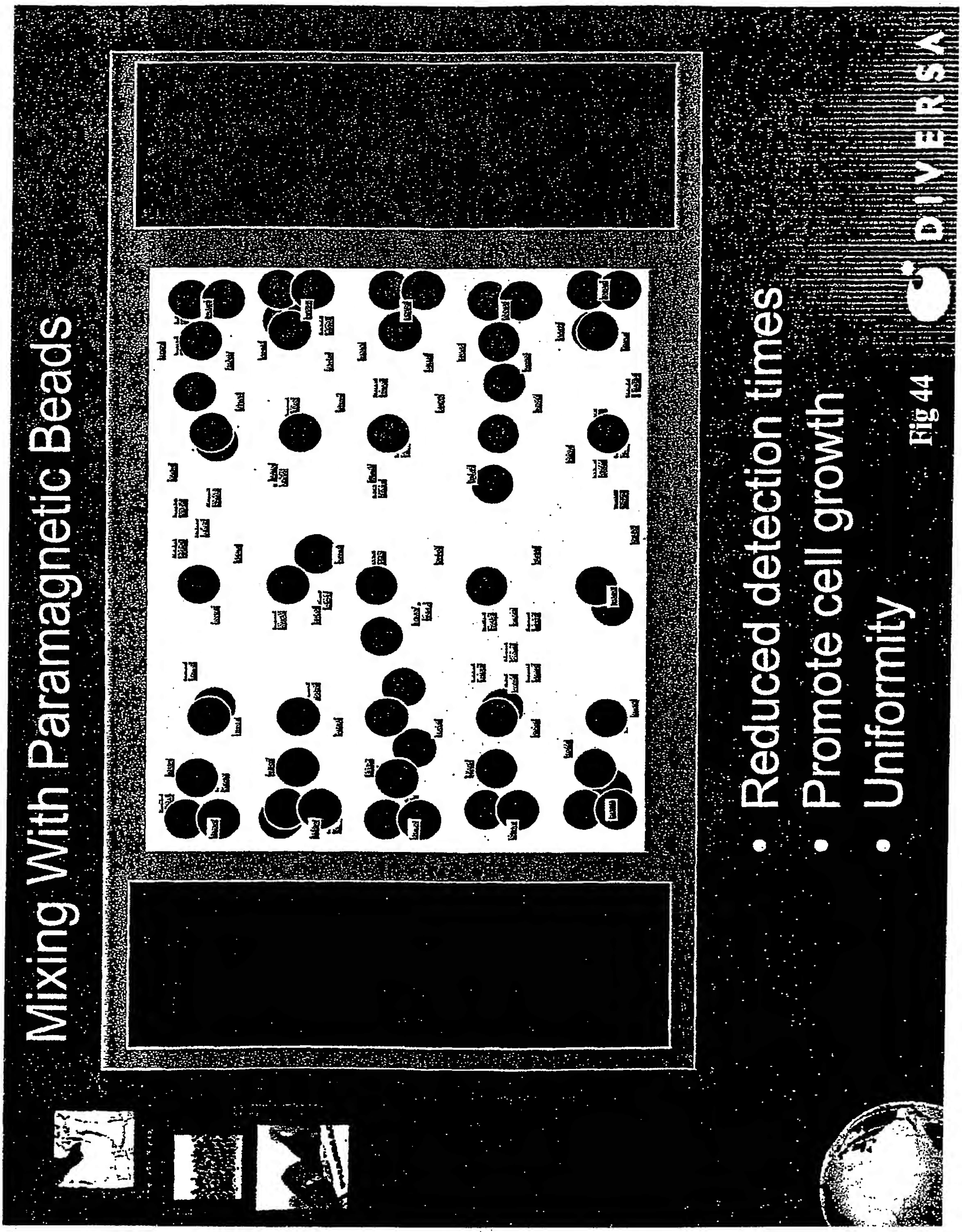
Mixing With Paramagnetic Beads
• Reduced detection times
• Promote cell growth
• Uniformity
DIVERSA

Fig 44

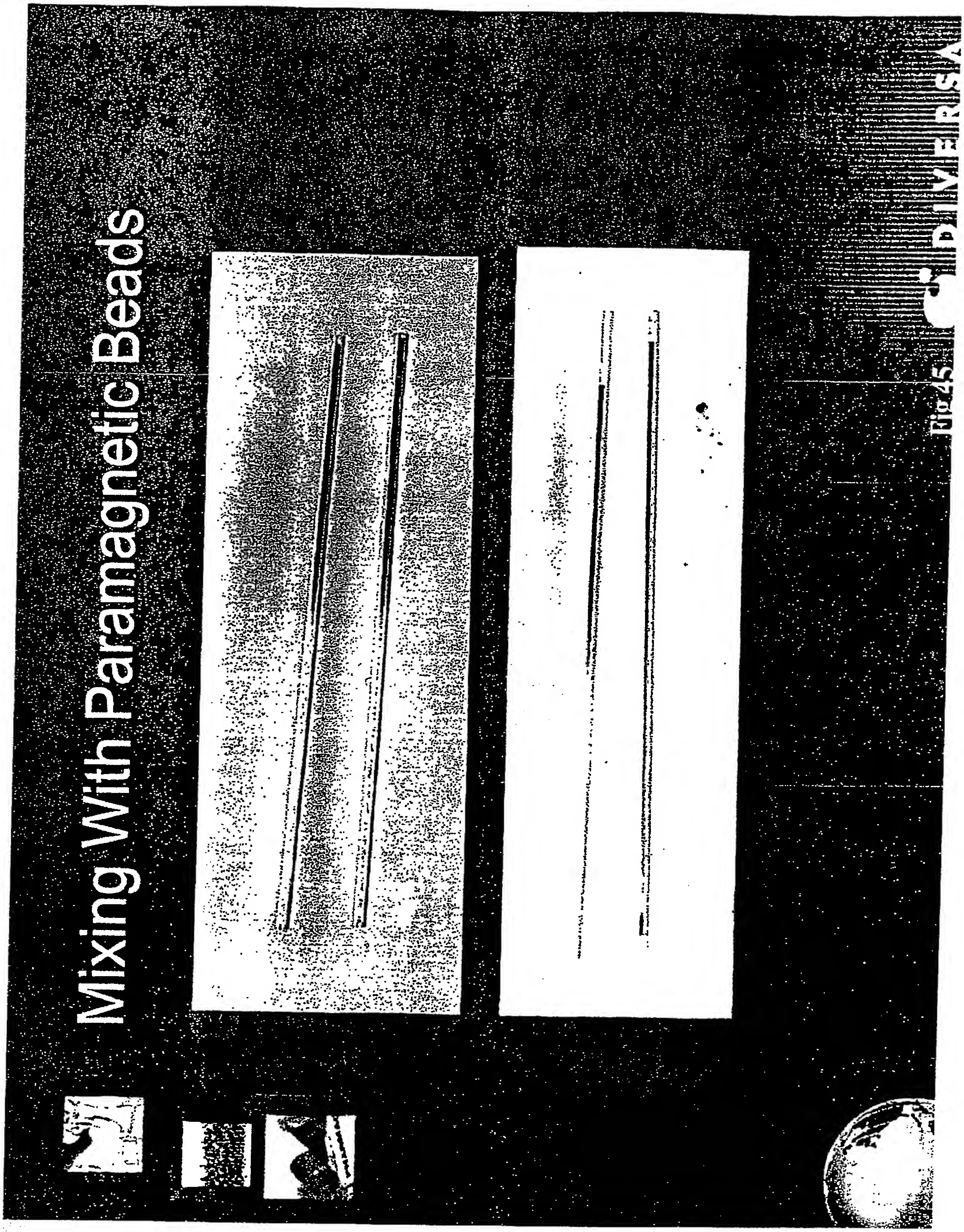

Fig 45

GigaMatrix™: Plate Density

Application	Well Diameter (µm)	Wells/ Plate	Volume* (nl)
Prototype	200	125,000	250
Nonlimiting Example: Mammalian	50	2,000,000	4
Nonlimiting Example: Bacterial	25	8,000,000	0.5
Nonlimiting Example: Process Limit	<5	128,000,000	0.007

* 40:1 length/diameter

Fig 46

DIVERSA

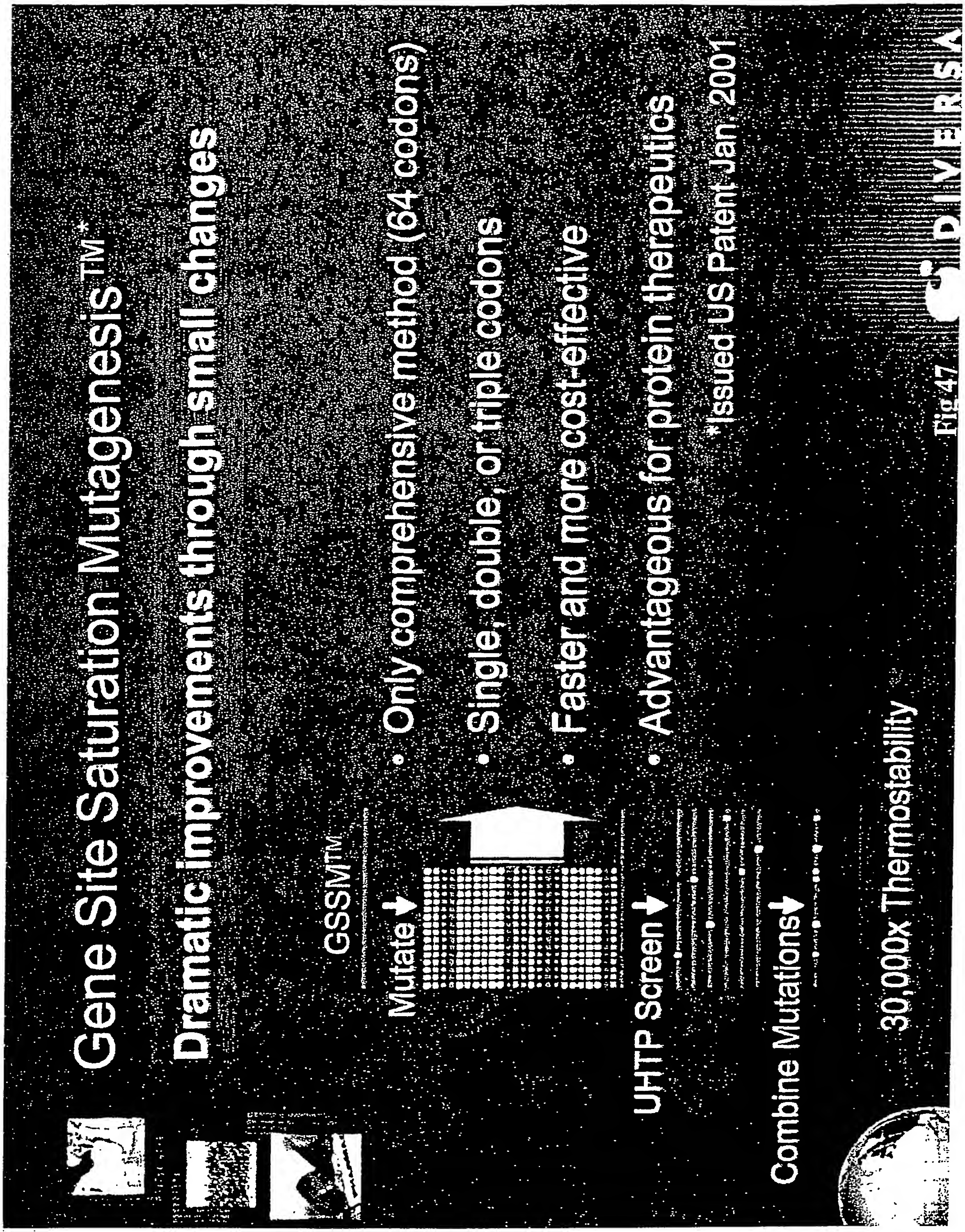
Gene Site Saturation Mutagenesis™*
Dramatic improvements through small changes
GSSM™
Mutate
UHTP Screen
Combine Mutations
30,000x Thermostability
• Only comprehensive method (64 codons)
• Single, double, or triple codons
• Faster and more cost-effective
• Advantageous for protein therapeutics
*Issued US Patent Jan. 2001
DIVERSA

Fig 47

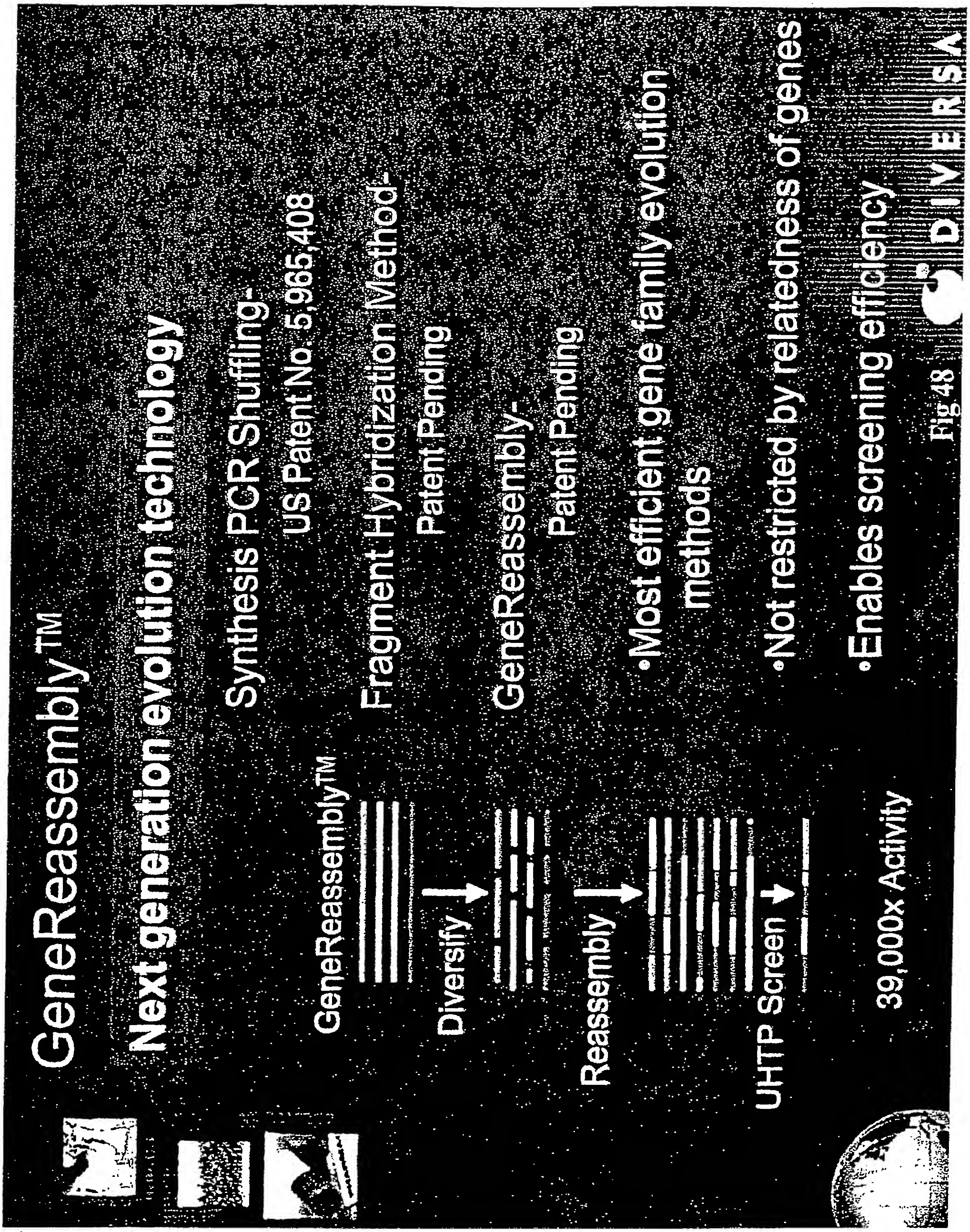
GeneReassembly™
Next generation evolution technology
GeneReassembly™
Diversify
Reassembly
UHTP Screen
39,000x Activity
Synthesis PCR Shuffling-
US Patent No. 5,965,408
Fragment Hybridization Method-
Patent Pending
GeneReassembly-
Patent Pending
•Most efficient gene family evolution methods
•Not restricted by relatedness of genes
•Enables screening efficiency
DIVERSA

Fig 48

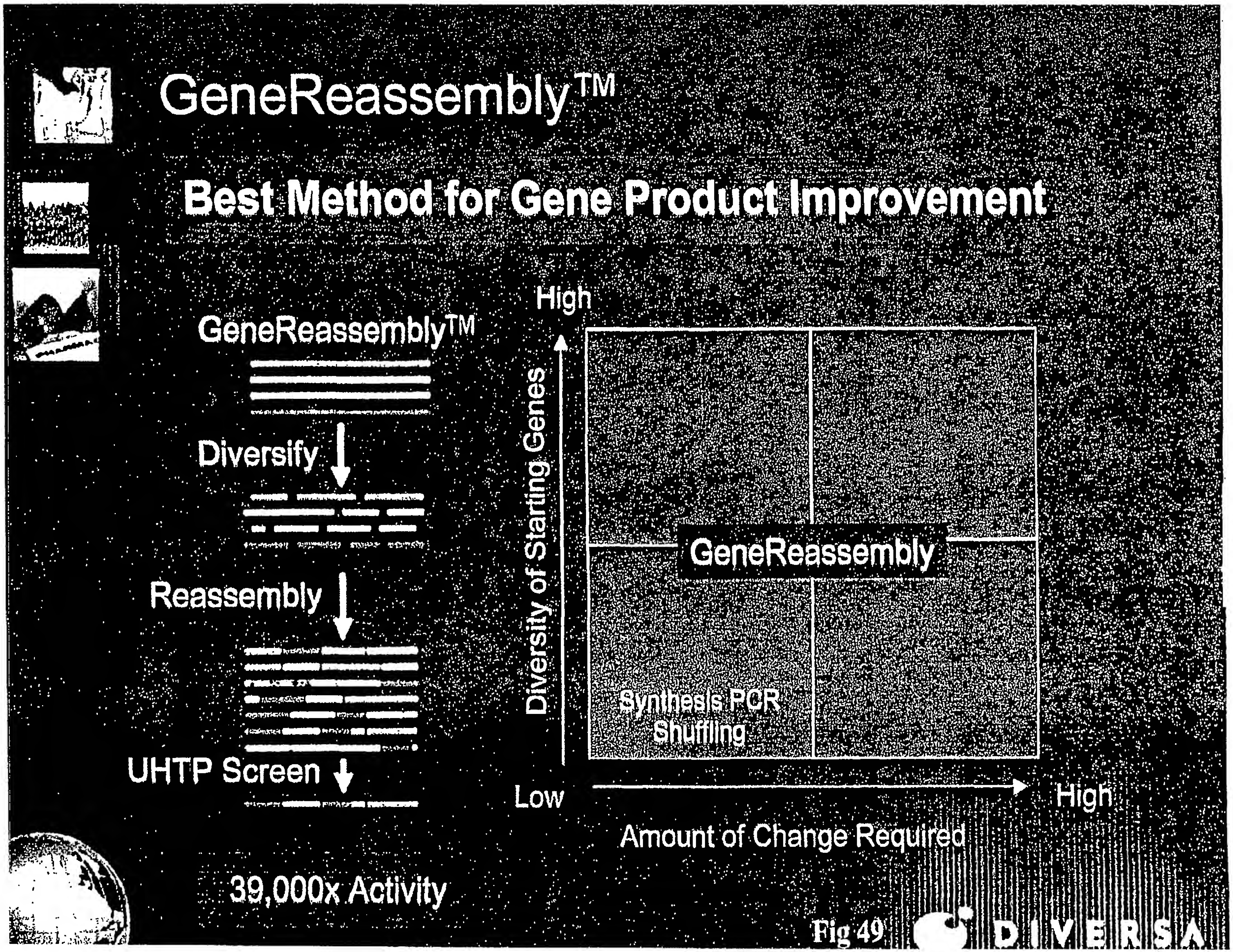
GeneReassembly™
Best Method for Gene Product Improvement
GeneReassembly™
Diversify
Reassembly
UHTP Screen
39,000x Activity
High
Diversity of Starting Genes
Low
GeneReassembly
Synthesis PCR
Shuffling
High
Amount of Change Required
DIVERSA

Fig 49

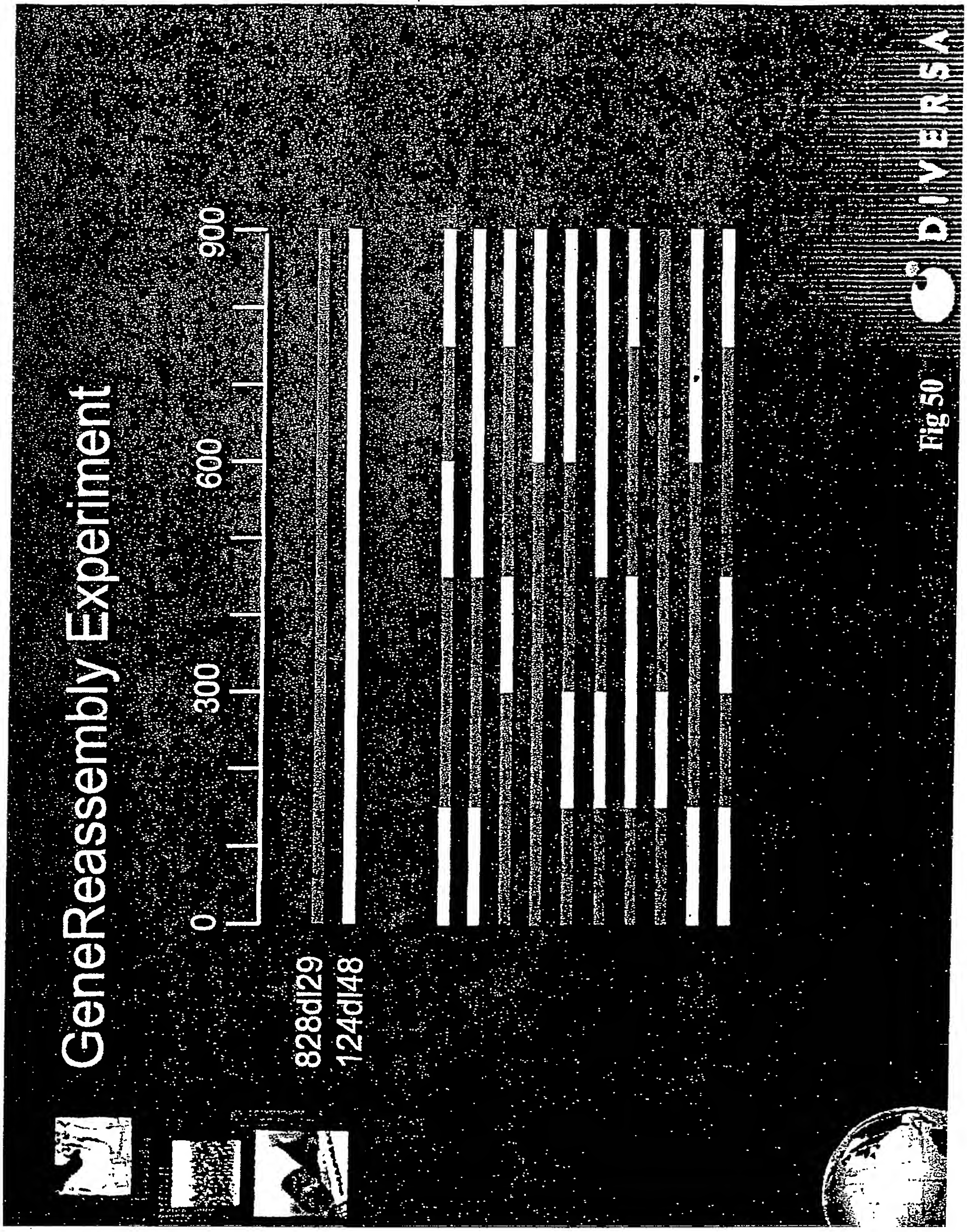

Fig 50

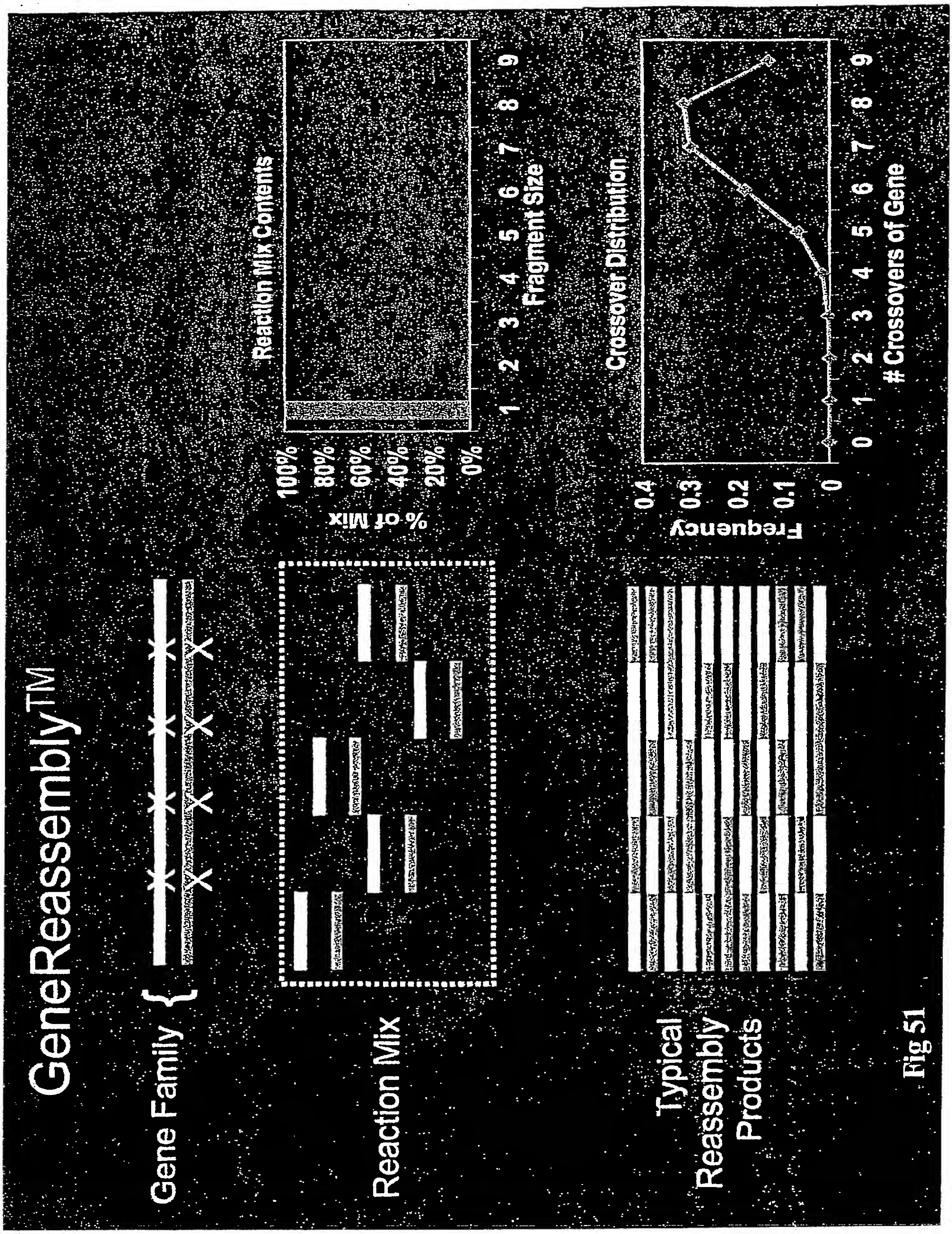
GeneReassembly™
Gene Family
Reaction Mix
Typical Reassembly Products
Reaction Mix Contents
% of Mix
100%
80%
60%
40%
20%
0%
1 2 3 4 5 6 7 8 9
Fragment Size
Crossover Distribution
Frequency
0.4
0.3
0.2
0.1
0
0 1 2 3 4 5 6 7 8 9
Crossovers of Gene

Fig 51

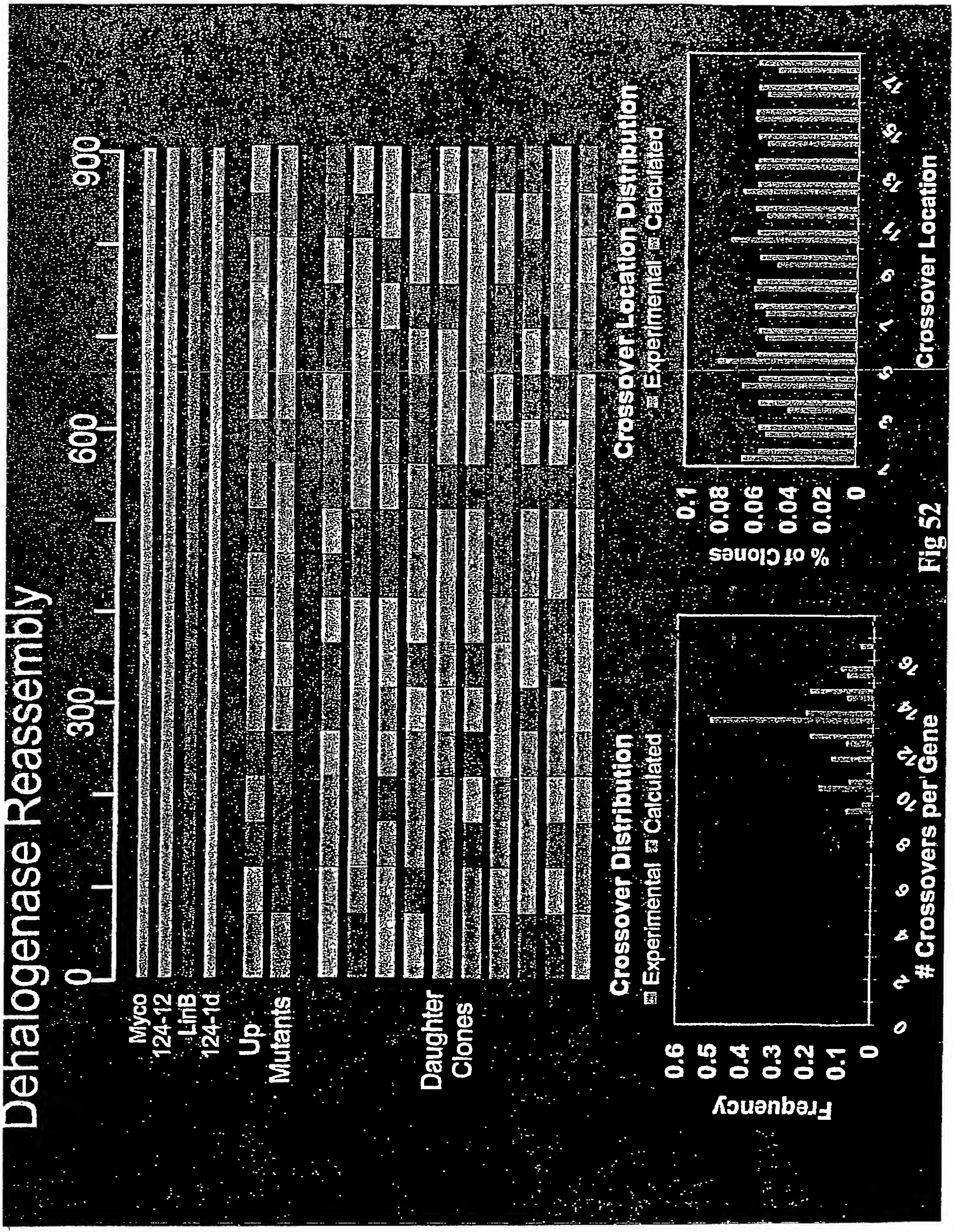
Dehalogenase Reassembly
0
300
600
900
Myco
124-12
LinB
124-1d
Up
Mutants
Daughter
Clones
Crossover Distribution
Experimental
Calculated
Frequency
0.6
0.5
0.4
0.3
0.2
0.1
0
0 2 4 6 8 10 12 14 16
Crossovers per Gene
Crossover Location Distribution
Experimental
Calculated
% of Clones
0.1
0.08
0.06
0.04
0.02
0
1 3 5 7 9 11 13 15 17
Crossover Location

Fig 52

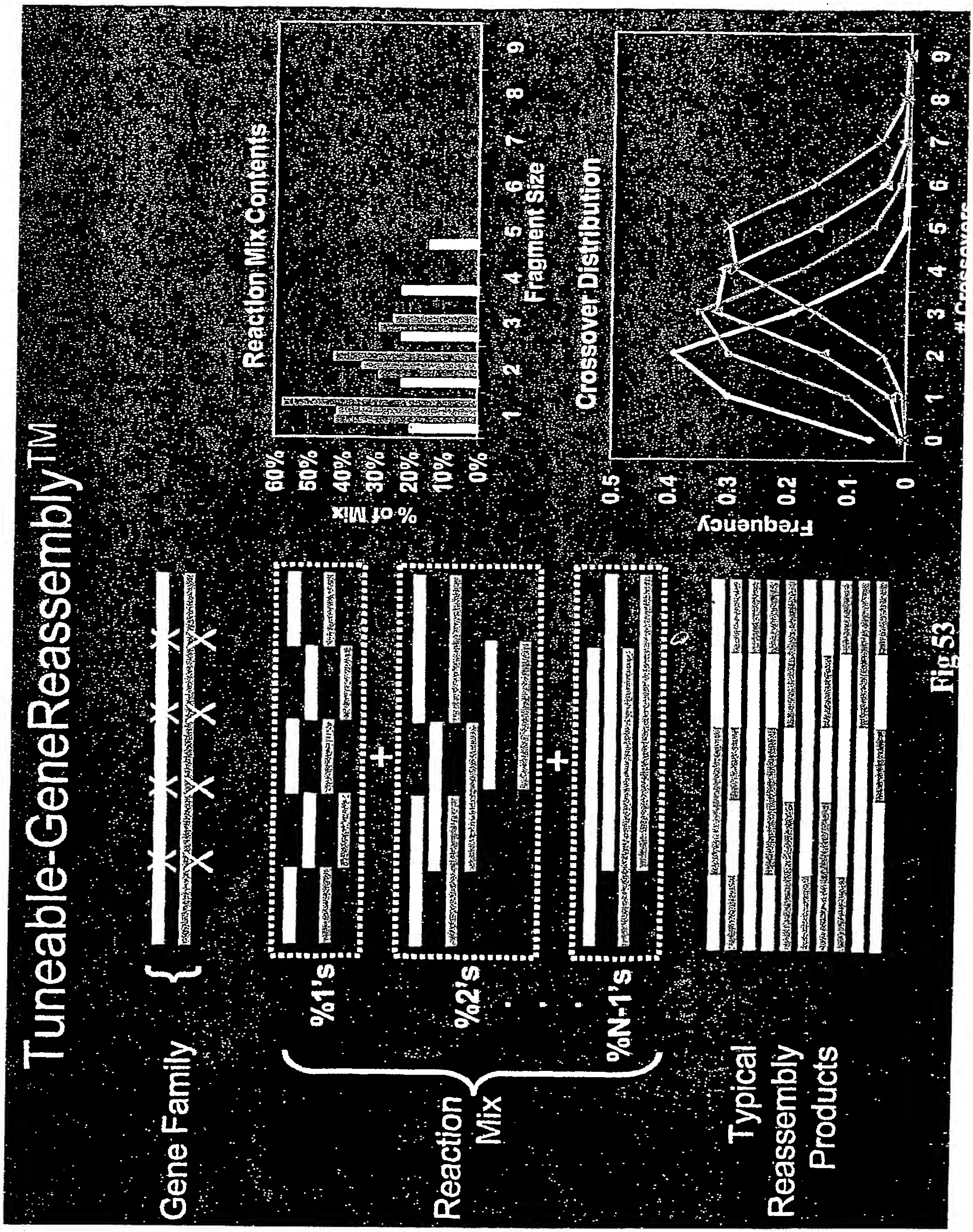
Tuneable-GeneReassembly™
Gene Family
Reaction Mix
%1's
%2's
%N-1's
Typical Reassembly Products
Reaction Mix Contents
60%
50%
40%
30%
20%
10%
0%
% of Mix
1 2 3 4 5 6 7 8 9
Fragment Size
Crossover Distribution
0.5
0.4
0.3
0.2
0.1
0
Frequency
0 1 2 3 4 5 6 7 8 9

Fig 53

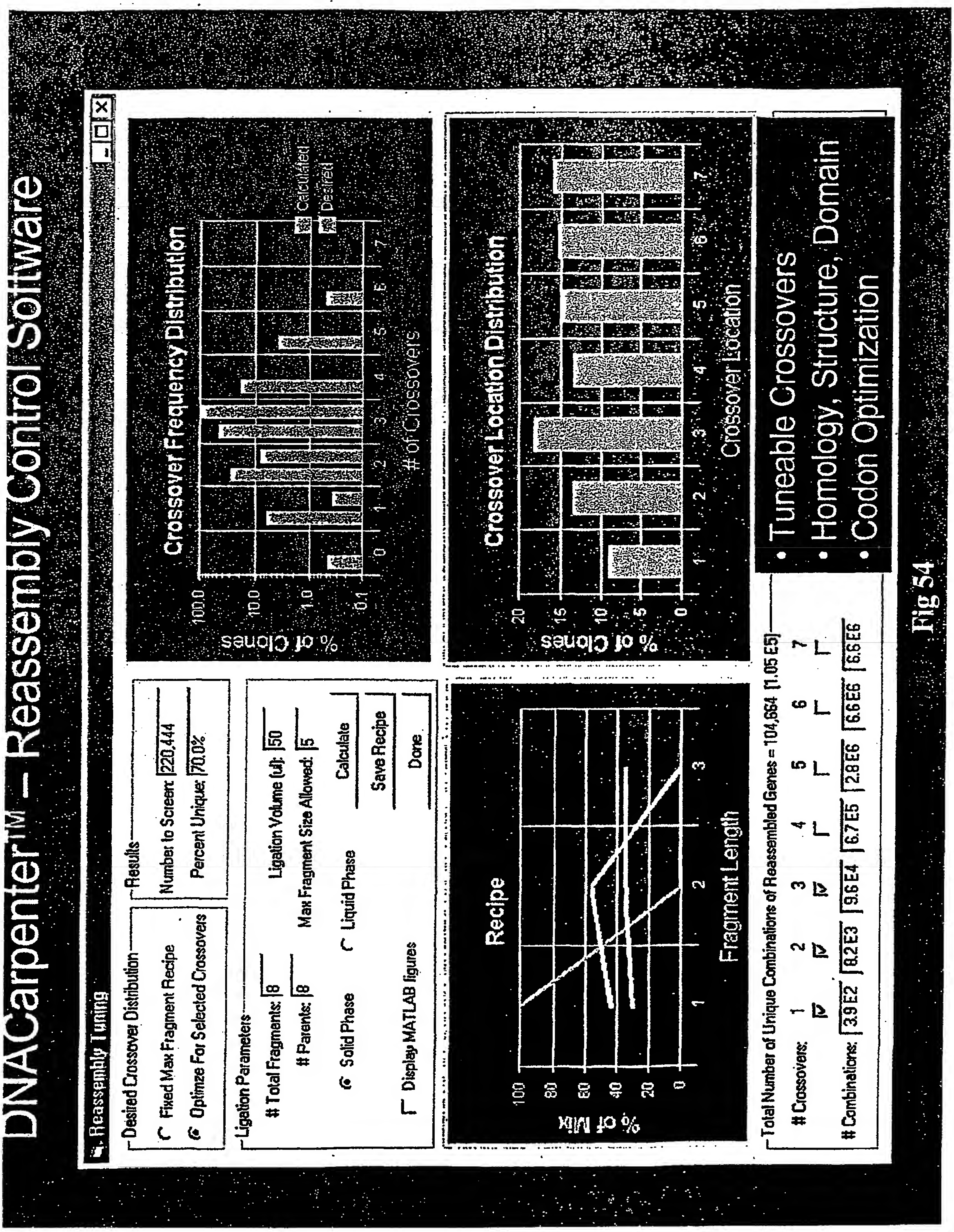
DNACarpenter™ – Reassembly Control Software
Reassembly Tuning
Desired Crossover Distribution
Fixed Max Fragment Recipe
Optimize For Selected Crossovers
Results
Number to Screen: 220,444
Percent Unique: 70.0%
Ligation Parameters
Total Fragments: 8
Parents: 8
Ligation Volume (ul): 50
Max Fragment Size Allowed: 5
Solid Phase
Liquid Phase
Calculate
Save Recipe
Done
Display MATLAB figures
Crossover Frequency Distribution
% of Clones
# of Crossovers
Calculated
Desired
Crossover Location Distribution
Crossover Location
Recipe
% of Mix
Fragment Length
Total Number of Unique Combinations of Reassembled Genes = 104,664 [1.05 E5]
Crossovers:
Combinations:
3.9E2
8.2E3
9.6E4
6.7E5
2.8E6
6.6E6
6.6E6
Tuneable Crossovers
Homology, Structure, Domain
Codon Optimization

Fig 54

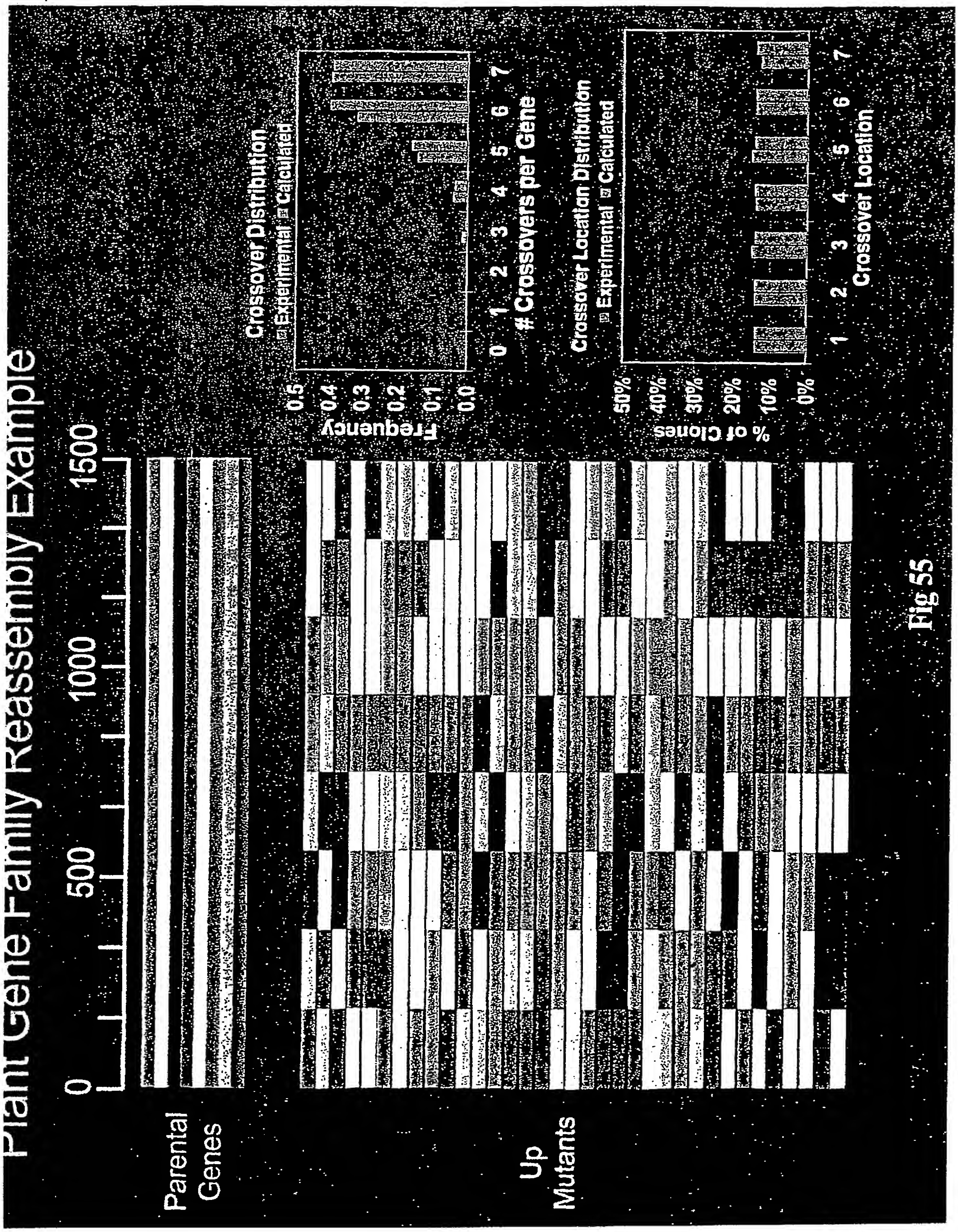
Plant Gene Family Reassembly Example
0
500
1000
1500
Parental Genes
Up Mutants
Crossover Distribution
Experimental Calculated
Frequency
0.5
0.4
0.3
0.2
0.1
0.0
0 1 2 3 4 5 6 7
Crossovers per Gene
Crossover Location Distribution
Experimental Calculated
% of Clones
50%
40%
30%
20%
10%
0%
1 2 3 4 5 6 7
Crossover Location

Fig 55

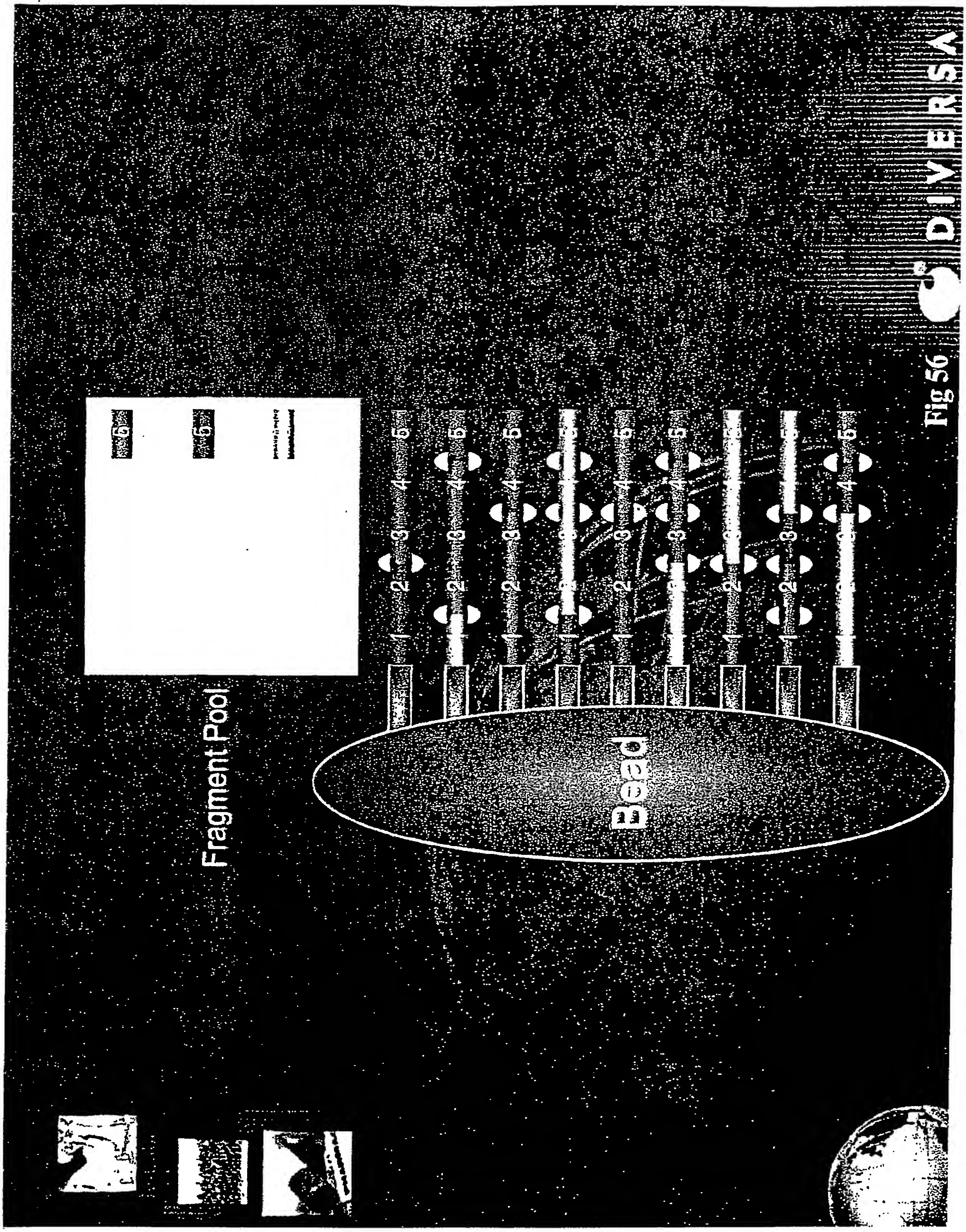

Fig 56

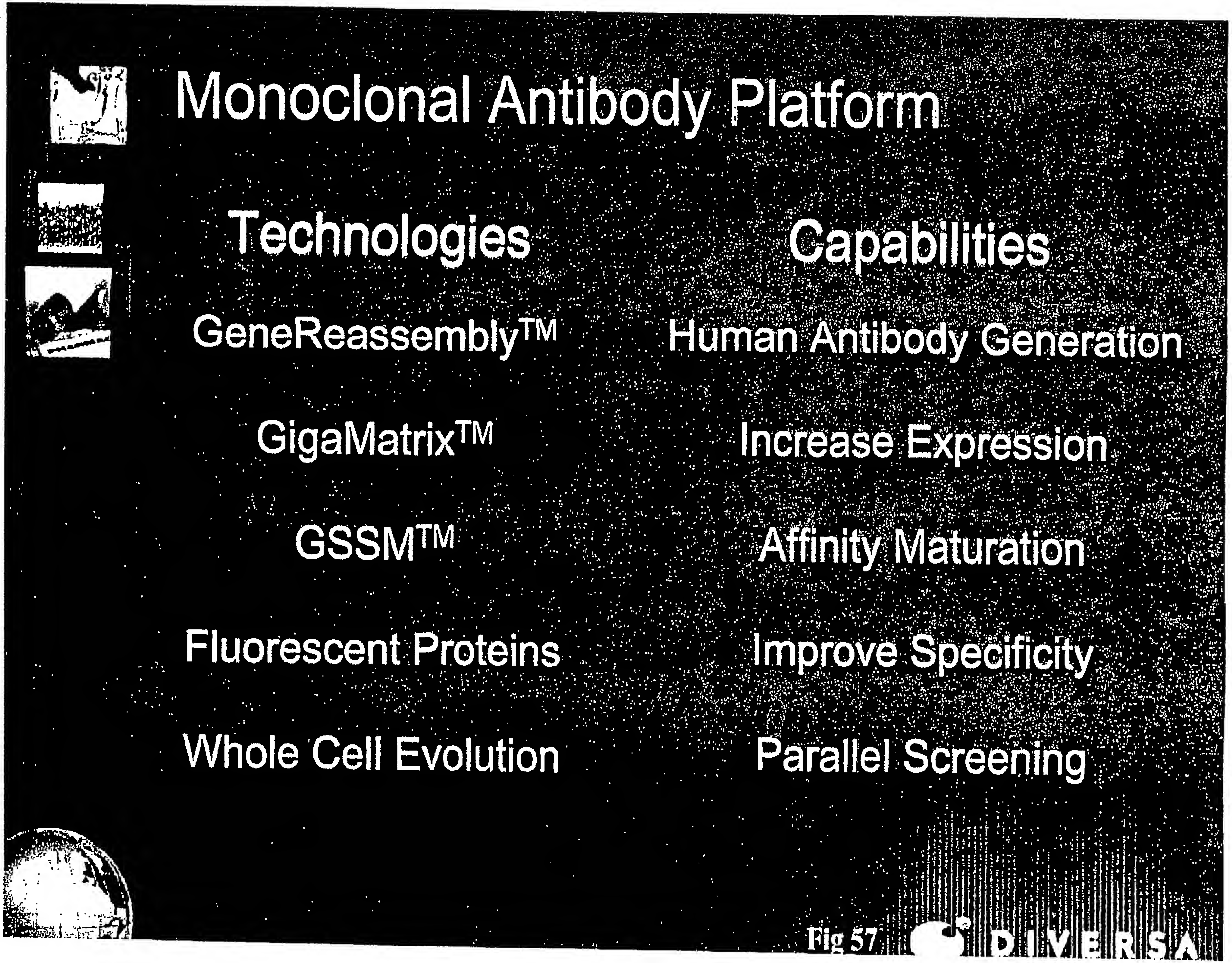
Monoclonal Antibody Platform
Technologies
GeneReassembly™
GigaMatrix™
GSSM™
Fluorescent Proteins
Whole Cell Evolution
Capabilities
Human Antibody Generation
Increase Expression
Affinity Maturation
Improve Specificity
Parallel Screening
DIVERSA

Fig 57

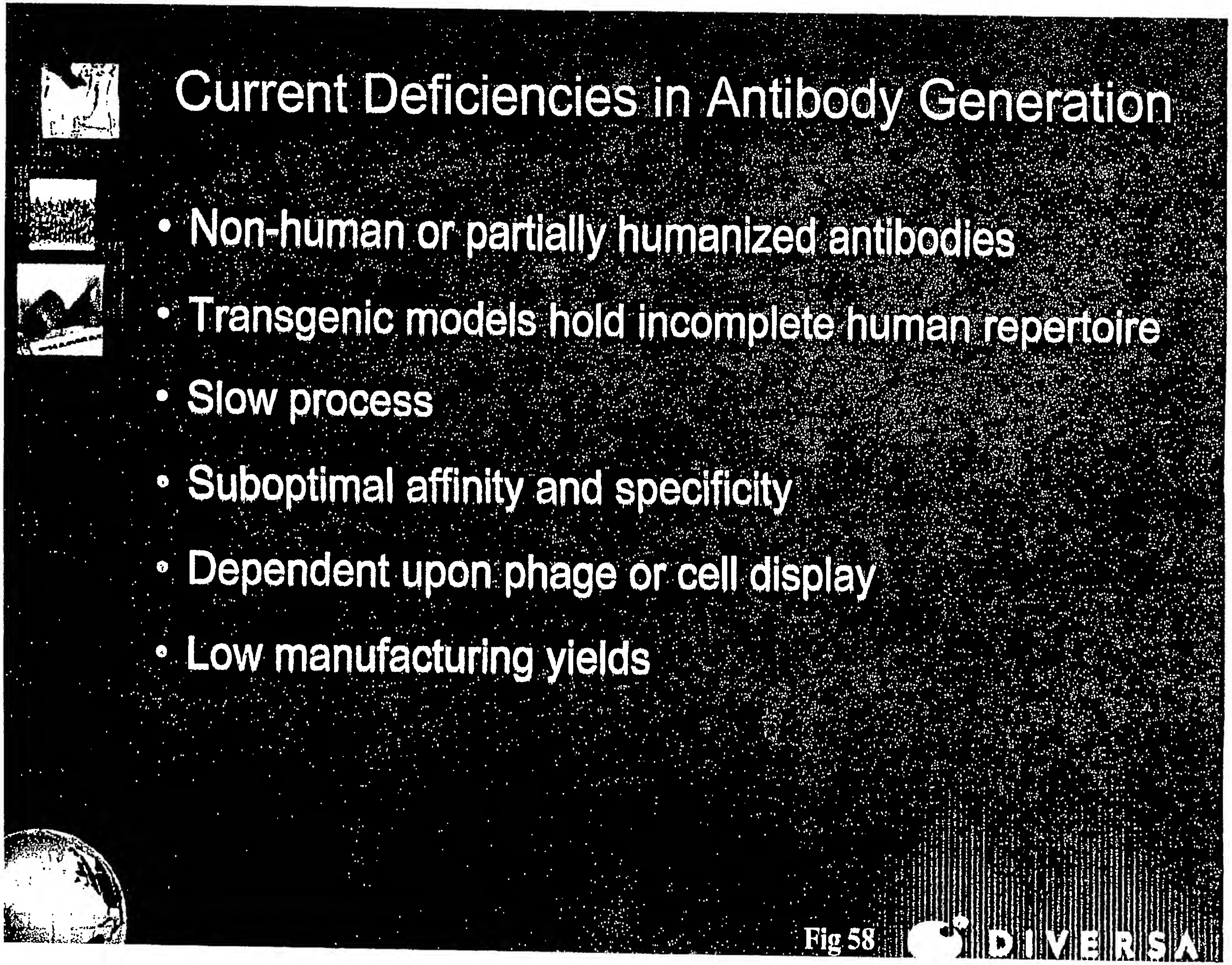
Current Deficiencies in Antibody Generation
• Non-human or partially humanized antibodies
• Transgenic models hold incomplete human repertoire
• Slow process
• Suboptimal affinity and specificity
• Dependent upon phage or cell display
• Low manufacturing yields
DIVERSA

Fig 58

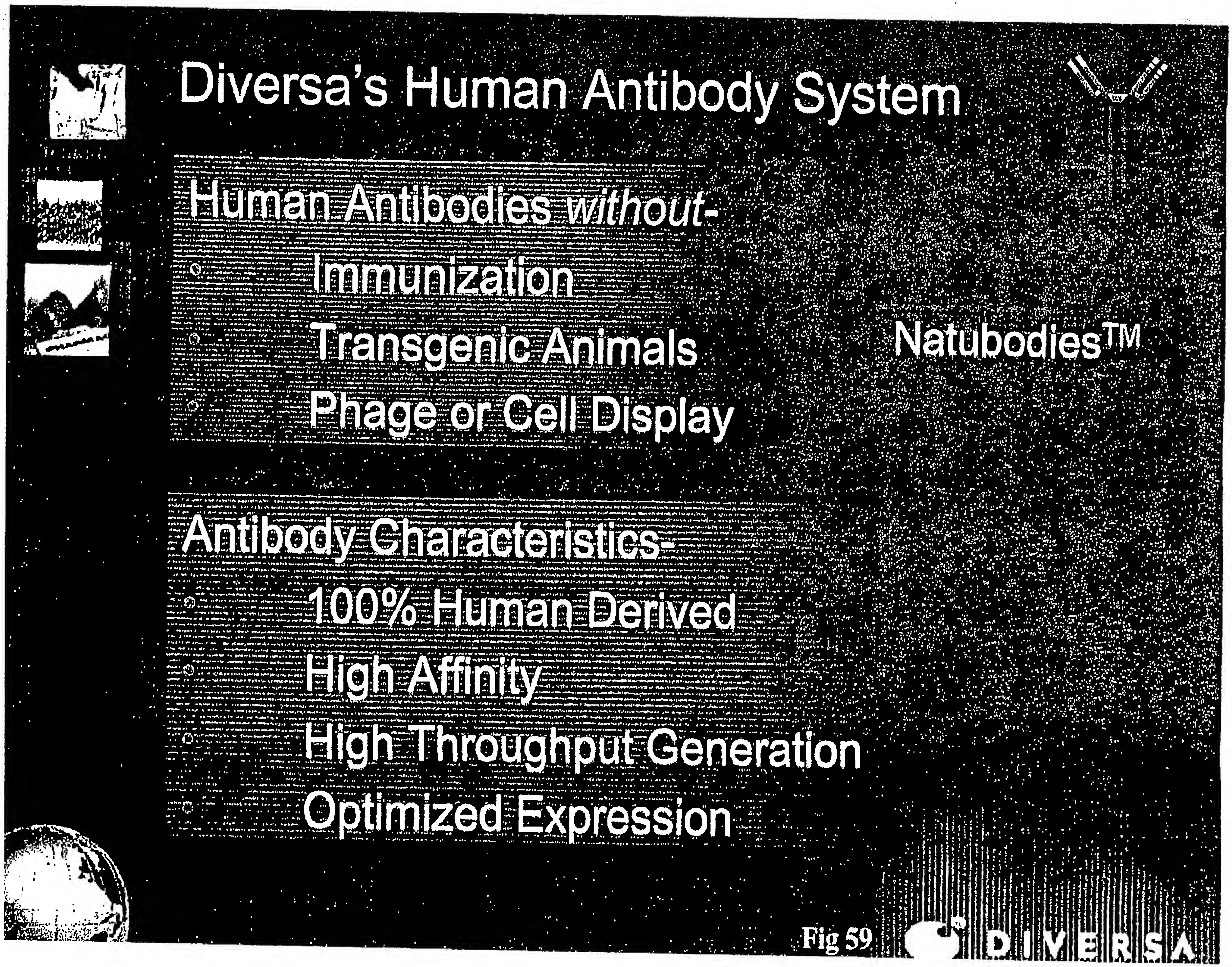
Diversa's Human Antibody System
Human Antibodies *without*-
Immunization
Transgenic Animals
Phage or Cell Display
Natubodies™
Antibody Characteristics-
100% Human Derived
High Affinity
High Throughput Generation
Optimized Expression
DIVERSA

Fig 59

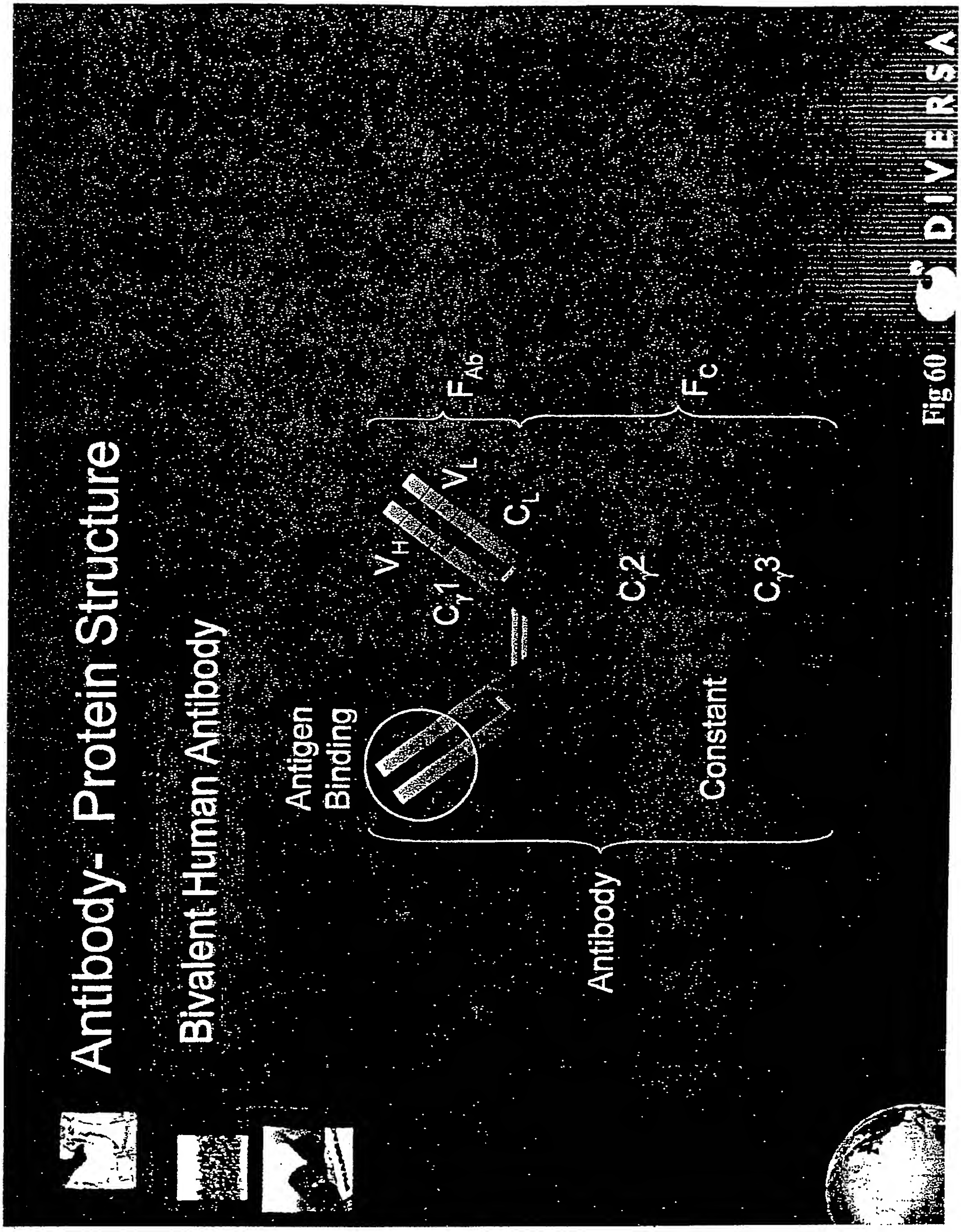
Antibody- Protein Structure
Bivalent Human Antibody
Antigen Binding
Constant
Antibody
V_H
V_L
C_L
$C_\gamma 1$
$C_\gamma 2$
$C_\gamma 3$
F_{Ab}
F_c
DIVERSA

Fig 60

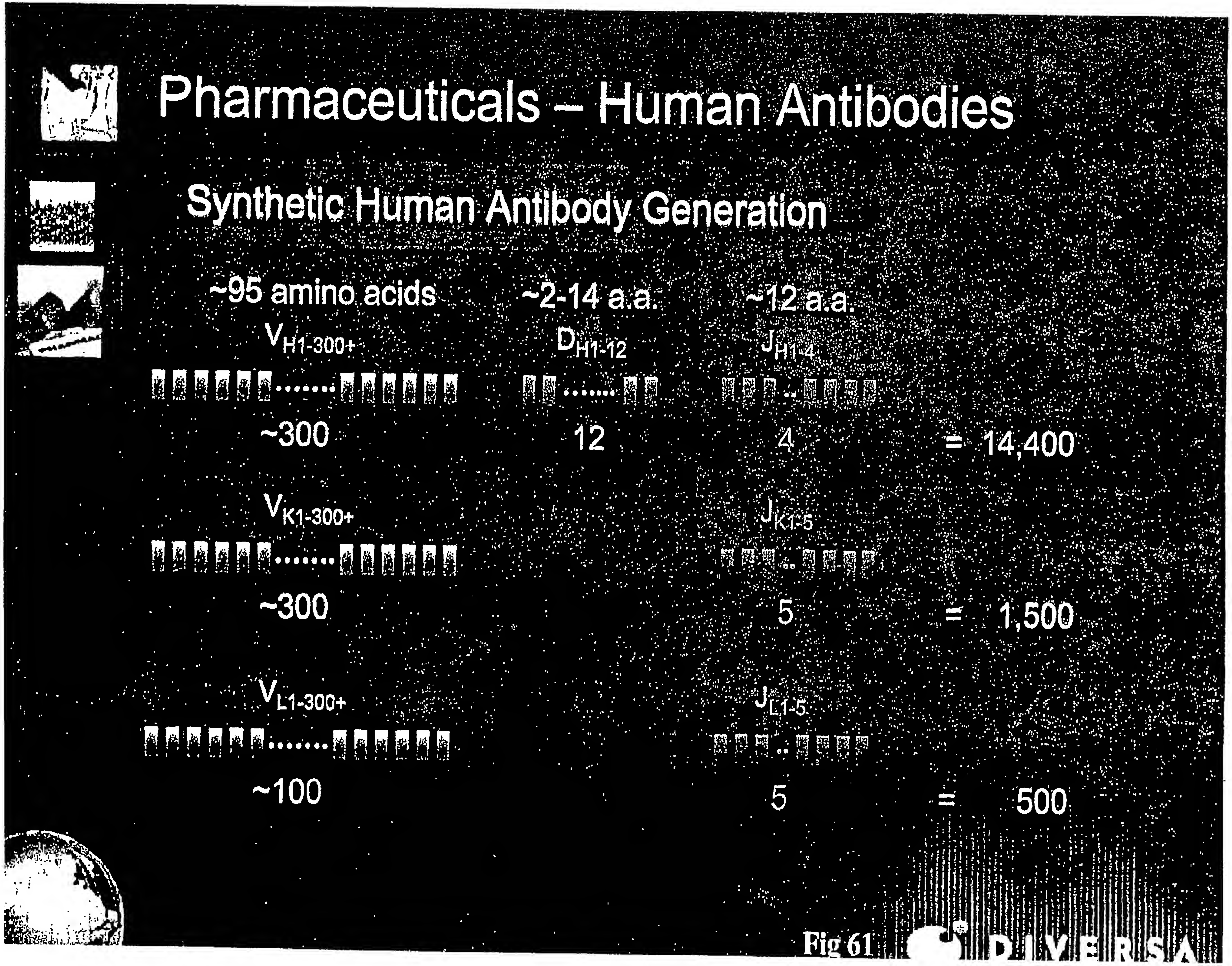
Pharmaceuticals – Human Antibodies
Synthetic Human Antibody Generation
~95 amino acids
~2-14 a.a.
~12 a.a.
$V_{H1\text{-}300+}$
$D_{H1\text{-}12}$
$J_{H1\text{-}4}$
~300
12
4
= 14,400
$V_{K1\text{-}300+}$
$J_{K1\text{-}5}$
~300
5
= 1,500
$V_{L1\text{-}300+}$
$J_{L1\text{-}5}$
~100
5
= 500
DIVERSA

Fig 61

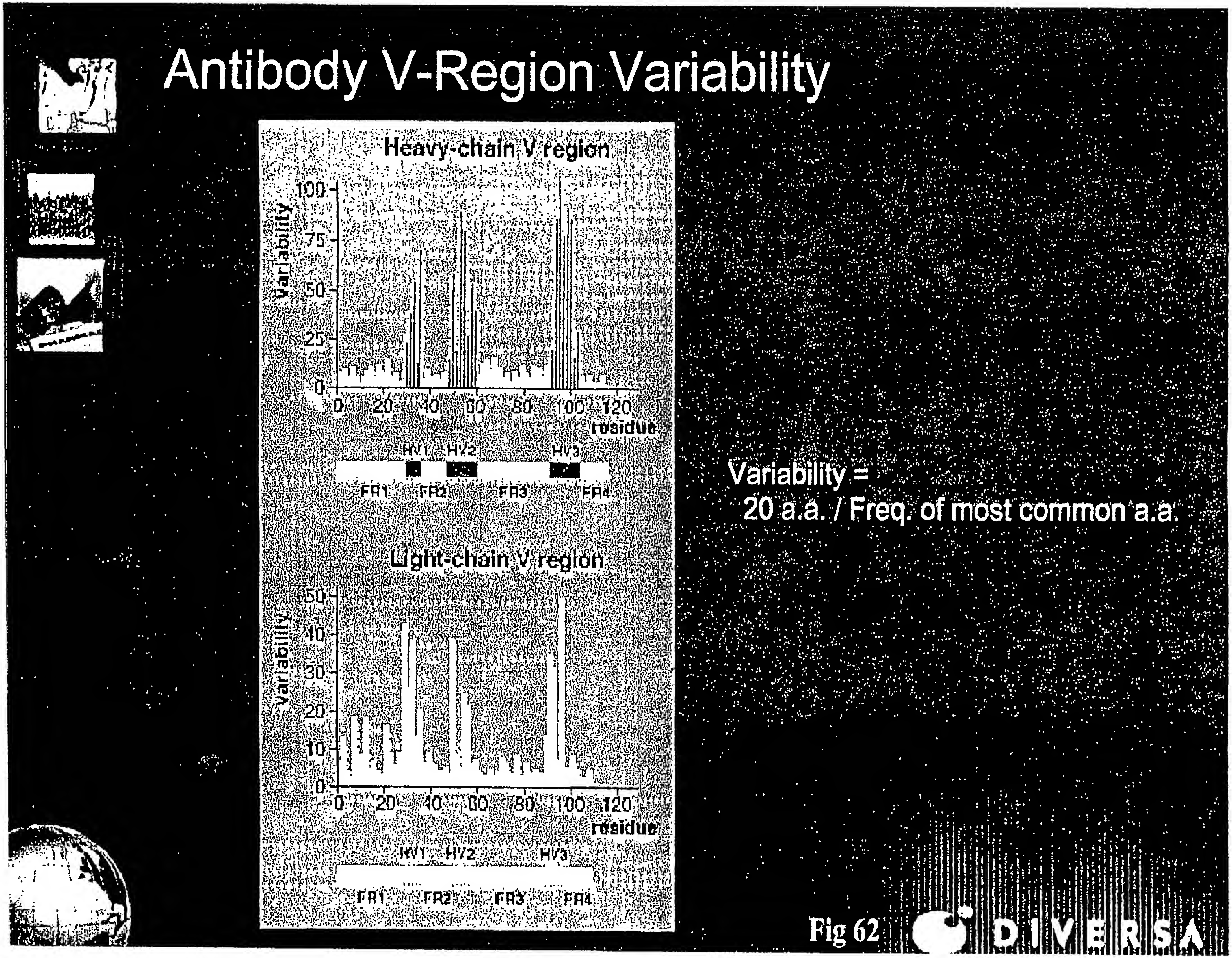
Antibody V-Region Variability
Heavy-chain V region
Variability
0 25 50 75 100
0 20 40 60 80 100 120
residue
HV1 HV2 HV3
FR1 FR2 FR3 FR4
Light-chain V region
Variability
0 10 20 30 40 50
0 20 40 60 80 100 120
residue
HV1 HV2 HV3
FR1 FR2 FR3 FR4
Variability =
20 a.a. / Freq. of most common a.a.
DIVERSA

Fig 62

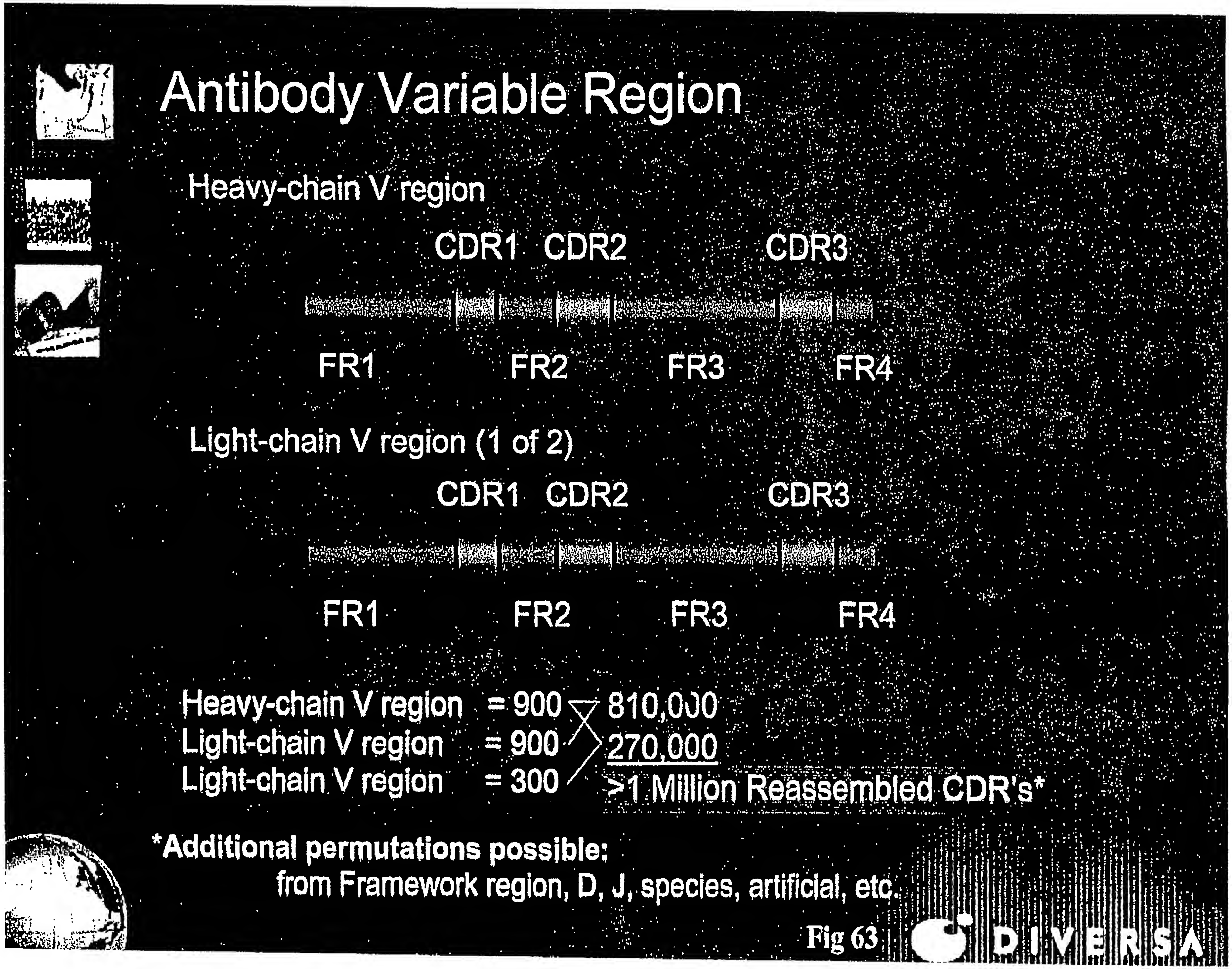
Antibody Variable Region
Heavy-chain V region
CDR1 CDR2 CDR3
FR1 FR2 FR3 FR4
Light-chain V region (1 of 2)
CDR1 CDR2 CDR3
FR1 FR2 FR3 FR4
Heavy-chain V region = 900
Light-chain V region = 900
Light-chain V region = 300
810,000
270,000
>1 Million Reassembled CDR's*
*Additional permutations possible:
from Framework region, D, J, species, artificial, etc.
DIVERSA

Fig 63

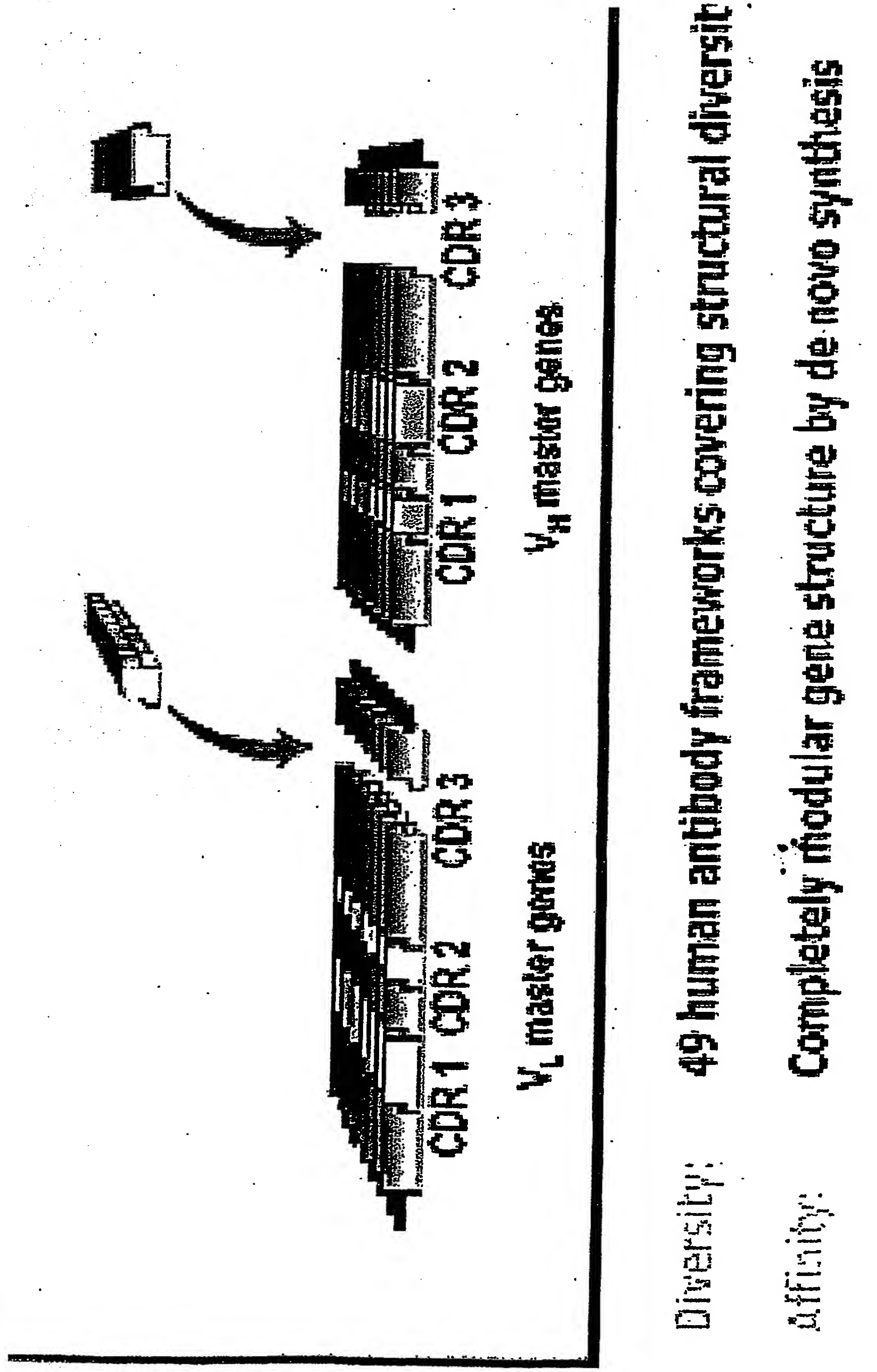
CDR 1 CDR 2 CDR 3
V_L master genes
CDR 1 CDR 1 CDR 2 CDR 3
V_H master genes
Diversity: 49 human antibody frameworks covering structural diversit
Affinity: Completely modular gene structure by de novo synthesis

Fig 64

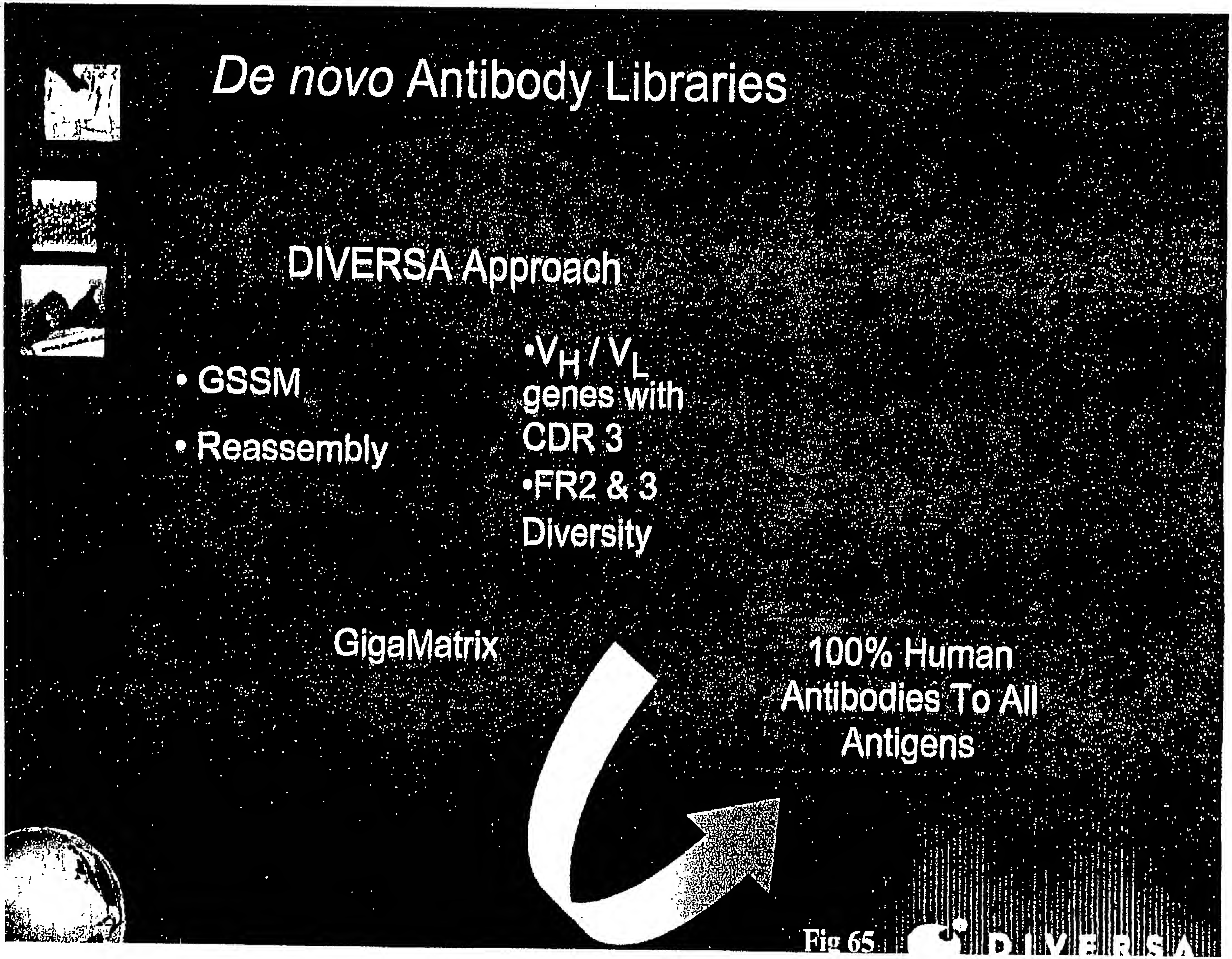
De novo Antibody Libraries
DIVERSA Approach
• GSSM
• Reassembly
•V_H / V_L genes with CDR 3
•FR2 & 3 Diversity
GigaMatrix
100% Human Antibodies To All Antigens
DIVERSA

Fig 65

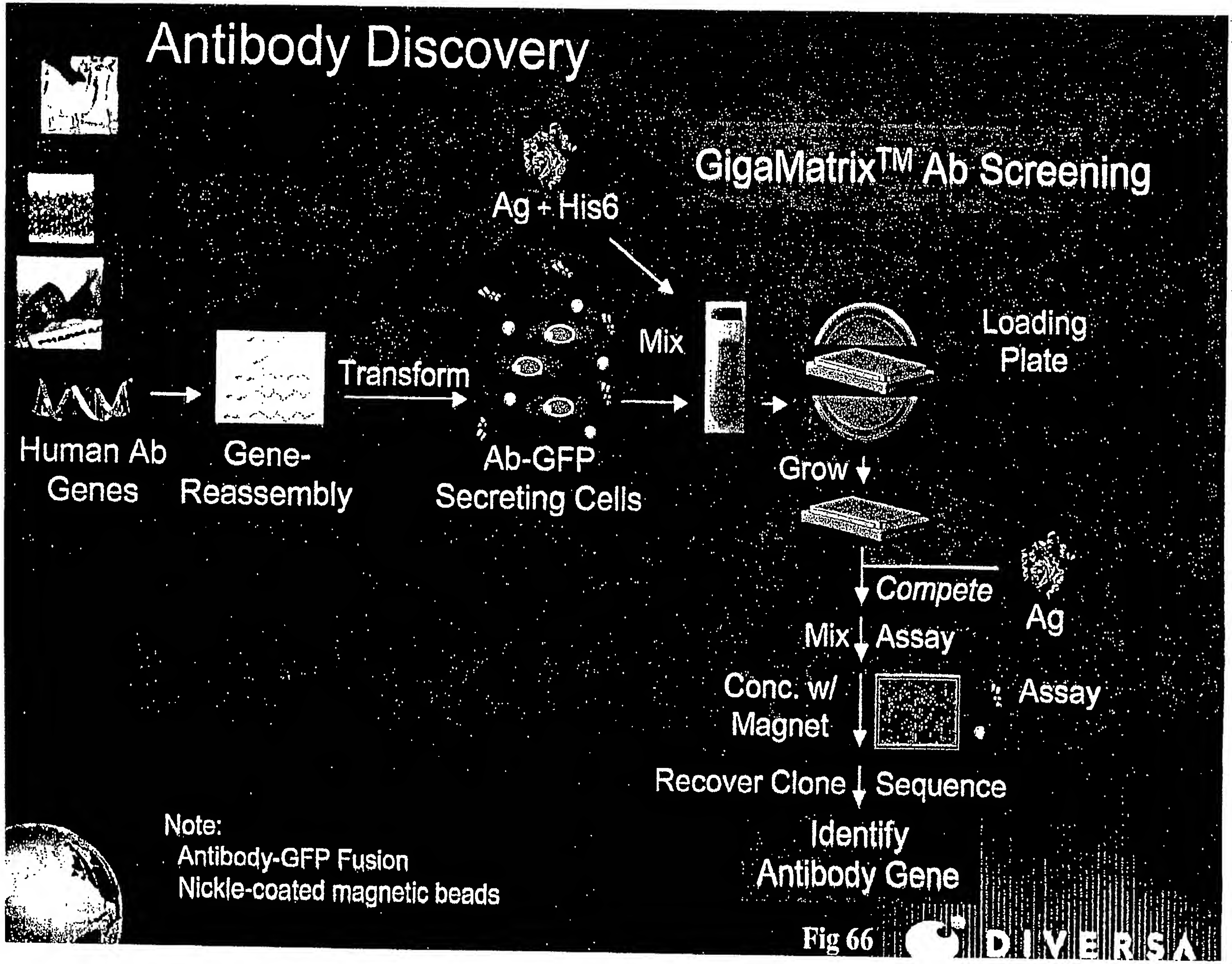
Antibody Discovery
GigaMatrix™ Ab Screening
Ag + His6
Human Ab Genes
Gene-Reassembly
Transform
Ab-GFP Secreting Cells
Mix
Loading Plate
Grow
Compete
Ag
Mix
Assay
Conc. w/ Magnet
Assay
Recover Clone
Sequence
Identify Antibody Gene
Note:
Antibody-GFP Fusion
Nickle-coated magnetic beads
DIVERSA

Fig 66

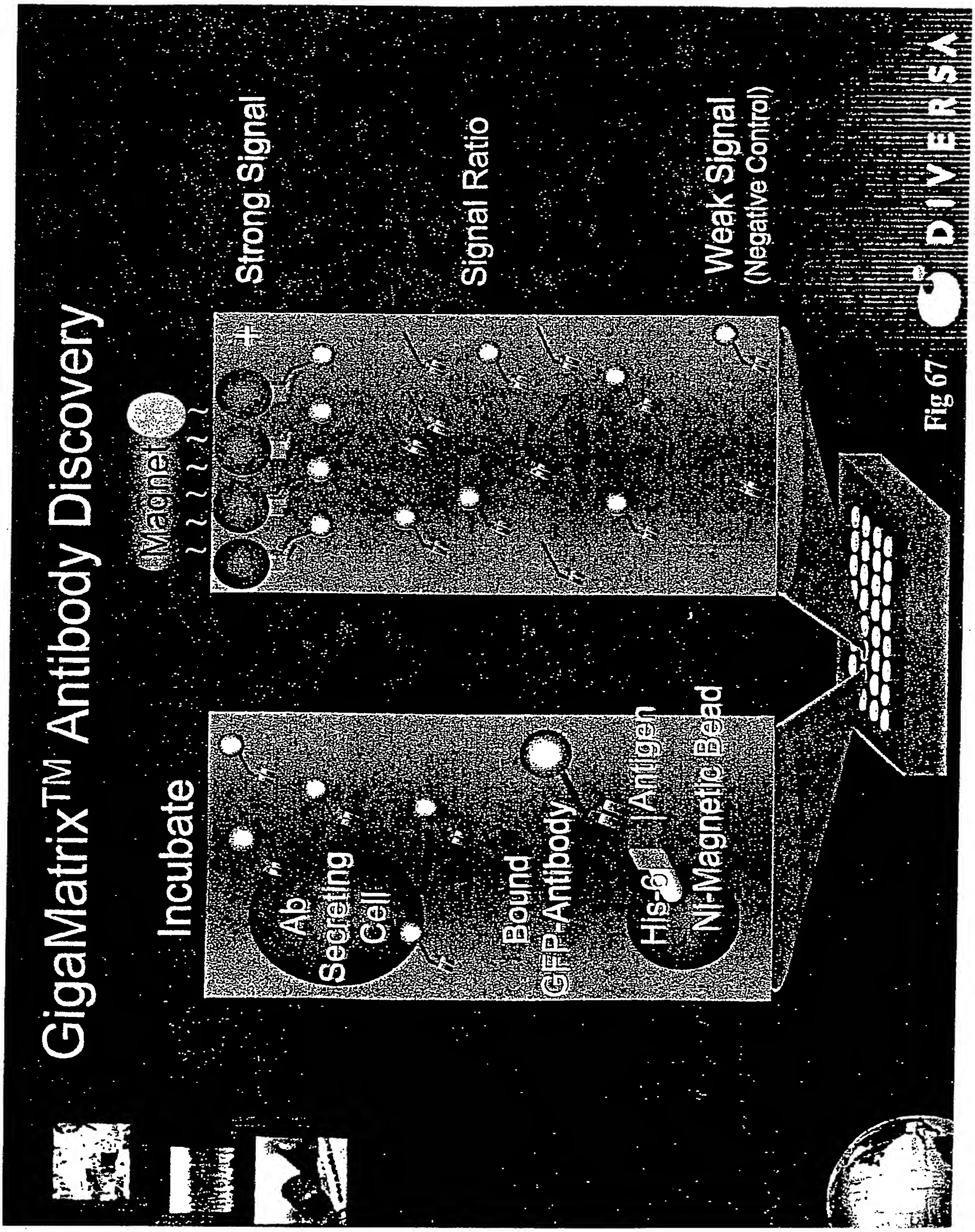
GigaMatrix™ Antibody Discovery
Incubate
Ab Secreting Cell
Bound GFP-Antibody
His-6
Antigen
Ni-Magnetic Bead
Magnet
Strong Signal
Signal Ratio
Weak Signal
(Negative Control)
DIVERSA

Fig 67

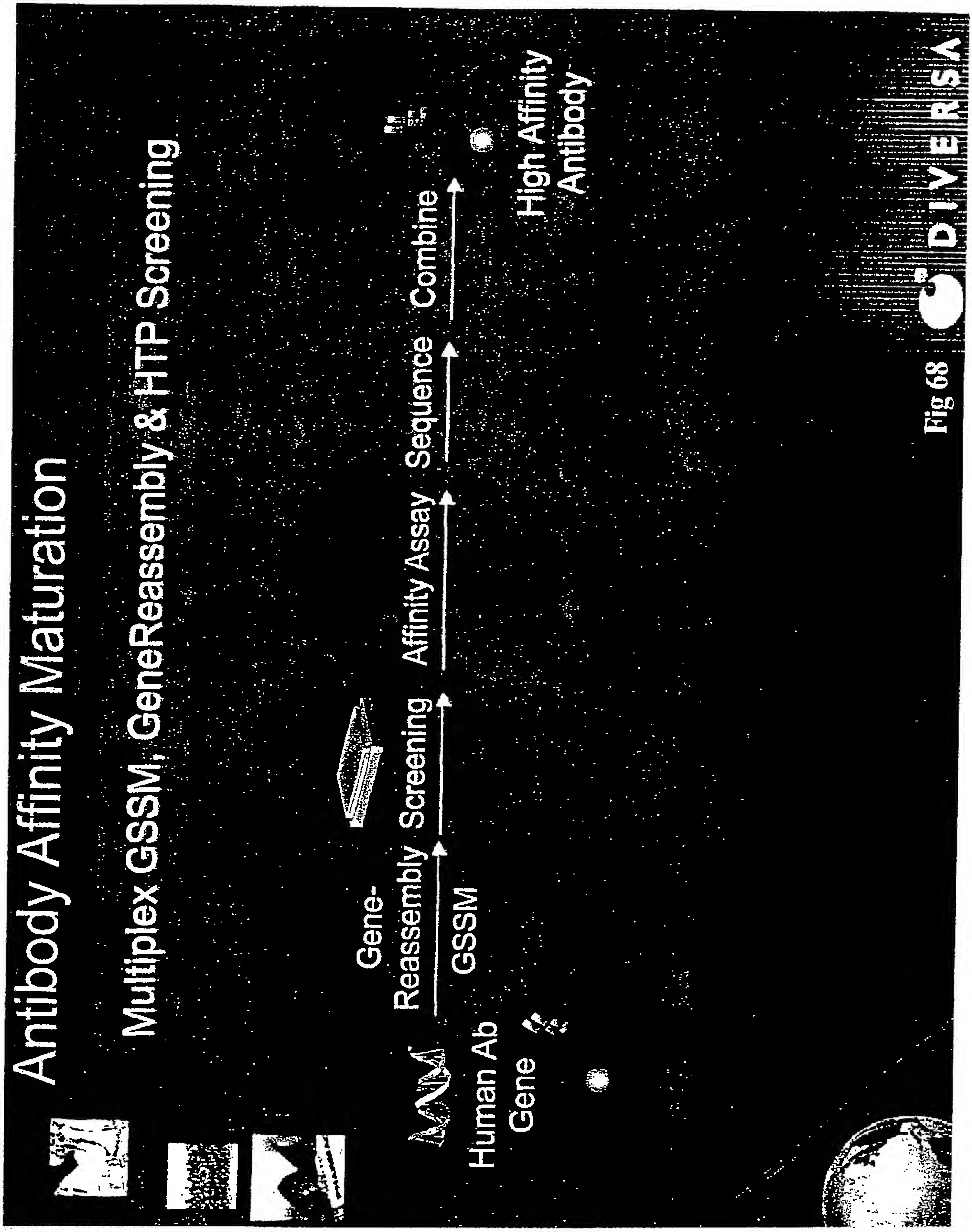
Antibody Affinity Maturation
Multiplex GSSM, GeneReassembly & HTP Screening
Human Ab Gene
Gene-Reassembly
GSSM
Screening
Affinity Assay
Sequence
Combine
High Affinity Antibody
DIVERSA

Fig 68

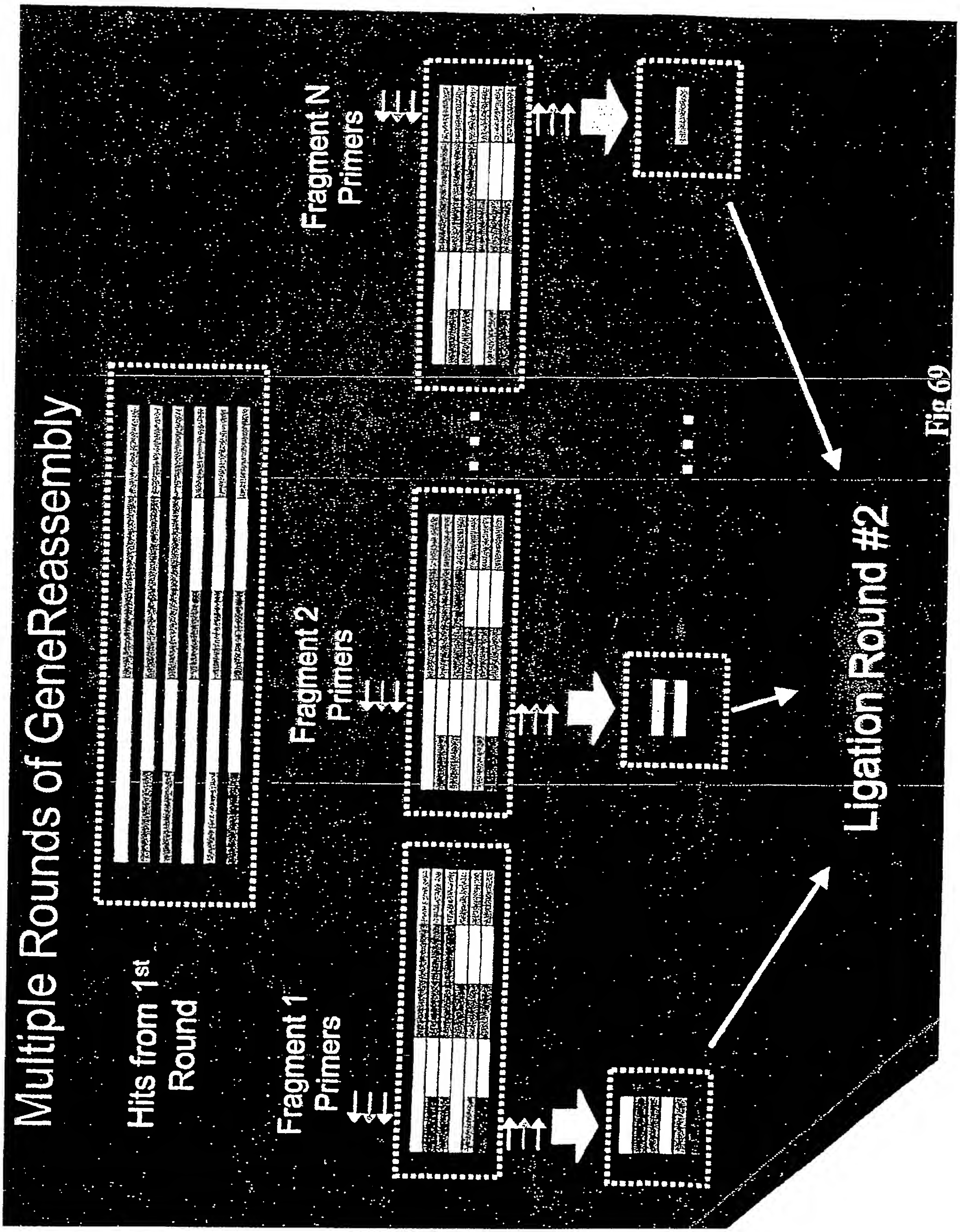
Multiple Rounds of GeneReassembly
Hits from 1st Round
Fragment 1 Primers
Fragment 2 Primers
Fragment N Primers
Ligation Round #2

Fig 69